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Thyroid Function and Risk of Anemia: A Multivariable-Adjusted and Mendelian Randomization Analysis in the UK Biobank

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Abbreviations: fT3, free triiodothyronine; fT4, free thyroxine; Hb, hemoglobin; HPT, hypothalamus-pituitary-thyroid; hs-CRP, high-sensitivity C-reactive protein; MR, Mendelian randomization; RTHα, resistance to thyroid hormone α; SNP, single nucleotide polymorphism; T3, triiodothyronine; T4, thyroxine; TR, thyroid hormone receptor; TSH, thyrotropin (thyroid-stimulating hormone); UKB, UK Biobank.

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Abstract

Context: Thyroid dysfunction is associated with higher anemia prevalence, although causality remains unclear.

Objective: This study aimed to investigate the association between thyroid function and anemia.

Methods: This cross-sectional and Mendelian randomization study included 445 482 European participants from the UK Biobank (mean age 56.77 years (SD 8.0); and 54.2% women). Self-reported clinical diagnosis of hypothyroidism was stated by 21 860 (4.9%); self-reported clinical diagnosis of hyperthyroidism by 3431 (0.8%). Anemia, defined as hemoglobin level of < 13 g/dL in men and < 12 g/dL in women, was present in 18 717 (4.2%) participants.

Results: In cross-sectional logistic regression analyses, self-reported clinical diagnoses of hypo- and hyperthyroidism were associated with higher odds of anemia (OR 1.12; 95% CI, 1.05-1.19 and OR 1.09; 95% CI, 0.91-1.30), although with wide confidence intervals for hyperthyroidism. We did not observe an association of higher or lower genetically influenced thyrotropin (TSH) with anemia (vs middle tertile: OR for lowest tertile 0.98 [95% CI, 0.95-1.02]; highest tertile 1.02 [95% CI, 0.98-1.06]), nor of genetically influenced

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free thyroxine (fT4) with anemia. Individuals with genetic variants in the *DIO3OS* gene implicated in intracellular regulation of thyroid hormones had a higher anemia risk (OR 1.05; 95% CI, 1.02-1.10); no association was observed with variants in *DIO1* or *DIO2* genes. **Conclusion:** While self-reported clinical diagnosis of hypothyroidism was associated with higher anemia risk, we did not find evidence supporting a causal association with variation of thyroid function within the euthyroid range. However, intracellular regulation of thyroid hormones might play a role in developing anemia.

Key Words: thyroid status, anemia, Mendelian randomization, UK Biobank

Anemia is a very common condition, affecting 27% of the world population (1). The definition of anemia is a deficiency of healthy erythrocytes, associated with reduced circulating levels of hemoglobin (Hb); the World Health Organization has set a universal threshold for anemia at a Hb level of < 13 g/dL in men and < 12 g/dL in women (2). Common causes of anemia include deficiency of iron, vitamin B12, and folate; chronic kidney disease; and inflammatory diseases (3, 4). Another possible cause for the development of anemia is an abnormal thyroid status (5).

Regulation of thyroid hormone availability is complex and occurs both centrally (hypothalamus-pituitary-thyroid [HPT] axis) and locally (by differential expression of thyroid hormone transporters, deiodinases, and nuclear thyroid hormone receptors, as well as transcriptional co-repressors and co-activators) (6). Under physiological conditions, both circulating thyroid-stimulating hormone (TSH) and free thyroxine (fT4) concentrations are regulated through negative feedback by the HPT axis. In target tissues, deiodinases can activate or deactivate thyroid hormones: deiodinase type 1 and 2 (D1 and D2) can convert the prohormone T4 into the active hormone triiodothyronine (T3), while deiodinase type 3 (D3) can convert T4 into inactive reverse T3 (7). The central regulation may fail in the presence of thyroid gland pathology, causing either hypothyroidism (biochemically characterized by low fT4 and elevated TSH) (8) or hyperthyroidism (characterized by elevated fT4 and low TSH) (9).

The production of sufficient numbers of differentiated red blood cells from hematopoietic progenitor cells is dependent on a delicate balance between proliferation and differentiation of progenitor cells. In addition to erythropoietin, interleukin 3, stem cell factor, and insulin-like growth factor 1, thyroid hormone has been implicated in erythropoiesis. Patients with resistance to thyroid hormone α (RTHα) have mild anemia. Also, animal studies suggest a role for thyroid hormone and thyroid hormone receptor (TR)α in erythropoiesis. Similarly, mice expressing a mutated TRα1 display anemia and reduced erythropoiesis. In wild-type mice, thyroid hormone stimulates the differentiation of progenitors to mature erythrocytes. In line with this, a reduction of mature erythrocytes was observed in TRα1 mutant mice (10). In addition, data from ex vivo cultures from RTHα patients suggest that thyroid hormone resistance results in a disturbed balance between proliferation and differentiation of erythrocytic progenitors, which may contribute to anemia (11). Multiple studies have suggested an association between thyroid dysfunction and anemia, including a possible U-shaped relationship between thyroid hormone levels and anemia (12), but causality of these associations remain to be proven (12-14). In Mendelian randomization (MR) studies, genetic variants associated with the exposure are used as instrumental variables free from most confounding and reverse causation (15). In the present MR study, we used genetic instruments for TSH and fT4 that were obtained in populations with participants within the euthyroid range as exposure, and anemia the outcome. For the weighted candidate gene analysis, we used genetic variants mapped to genes encoding deiodinases which were identified in recent genome-wide association studies (GWAS) (16) as associated with circulating fT4 levels. We therefore hypothesized that these variants may have functional implications in local thyroid hormone homeostasis.

Methods

To investigate whether the relationship between thyroid function and anemia is causal, we used a multi-approach strategy and performed cross-sectional multivariable-adjusted logistic regression, Mendelian randomization (MR), and weighted candidate gene analyses. Multivariable-adjusted regression analyses were conducted to replicate previous studies regarding the association of hypo- and hyperthyroidism and anemia.

Study Population

The UK Biobank (UKB) cohort is a prospective general population cohort. Baseline assessments took place between 2006 and 2010 in 22 different assessment centers across the United Kingdom (17). A total of 502 628 participants aged

between 40 and 70 years were recruited from the general population. Invitation letters were sent to eligible adults registered to the National Health Services (NHS) and living within 25 miles from one of the study assessment centers. The response rate was 5.5% (18). At the study assessment center, participants completed touchscreen-based questionnaires that included topics such as sociodemographic characteristics, physical and mental health, lifestyle, and habitual food intake.

For the present study, we selected all individuals with a self-reported European ancestry, available genomics data, and data on Hb levels. As a result, we included a total sample of 445 482 individuals. The UK Biobank has approval from the NHS North West Multi-Centre Research Ethics Committee (ref 11/NW/0382). All participants from the UKB cohort provided written informed consent, and the study was approved by the medical ethics committee. The current project was completed under project number 32743.

Exposure Assessment

Self-reported clinical diagnosis of hypo- or hyperthyroidism

At the assessment center, UKB participants were asked in a verbal interview by a trained research nurse whether they had either a clinical diagnosis of hypo- or hyperthyroidism, and whether they used any medication for thyroid dysfunction (17). Within this study, liothyronine (T3 suppletion), levothyroxine (T4 suppletion), antithyroid medication or a combination therapy were considered as thyroid medication (ATC code H03).

Selection of the genetic instruments for TSH and fT4 for the Mendelian randomization

We selected all independently associated single nucleotide polymorphisms (SNPs) (P value $< 5 \times 10^{-8}$ (15)) for circulating TSH and fT4 levels within the reference range as instrumental variables from a previously performed genome-wide association meta-analysis (16). Study-specific protocols of the studies contributing to the meta-analysis are described previously (16). This genome-wide association meta-analysis, being the largest conducted in European-ancestry participants on TSH and fT4 concentrations in the euthyroid range, was performed in 71 167 European-ancestry participants from 22 different cohorts (16). From this effort, we derived a total of 62 SNPs associated with TSH (explaining 9.4% of the total variance in TSH level (19)) and 31 SNPs associated with fT4 (explaining 4.8% of the total variance in fT4 level (19)). Based on the SNP effect sizes, we calculated weighted genetic risk scores for each individual included in the study.

Genetic risk scores based on deiodinase activity

To explore the role of deiodinase activity in anemia implicated in intracellular regulation of thyroid hormones, we calculated separate genetic risk scores based on fT4-associated SNPs mapped to *DIO1*, *DIO2*, and *DIO3OS*. These scores were weighted to the association of each SNP with circulating fT4, as a surrogate for the magnitude of the intracellular effects.

Anemia

For measuring blood Hb levels, blood was collected into a 4-mL EDTA vacutainer and held in temperature-controlled shipping boxes (4 °C). Complete blood cell counts were conducted using a Coulter counter. The universal definition of anemia in adults is an Hb level of < 13 g/dL in men and < 12 g/dL in women, as proposed by the World Health Organization (2).

Covariates

Low-grade systemic inflammation was considered a possible important confounder for the analyses with self-reported clinical diagnosis of hypo- and hyperthyroidism, since inflammation affects both thyroid status (20) and risk of anemia (21) via distinct biological pathways. These analyses were therefore adjusted for factors related to low-grade systemic inflammation, notably levels of high-sensitivity C-reactive protein (hs-CRP) and the lifestyle factors smoking and alcohol intake. Smoking status was assessed via a touchscreen questionnaire inquiring whether the participant was currently smoking, past smoker, or never smoker; for the present study we dichotomized smoking status into current smoker or nonsmoker. Alcohol intake was also assessed by touchscreen questionnaire. All participants were asked how frequently they drank alcoholic beverages. As there is no guideline on frequency of drinking alcoholic beverages, we aimed to divide into 2 groups of similar size that could be interpreted as drinking more or less frequently than average. For the present population the cutoff point was up to 2 times per week or more than twice a week.

Statistical Analyses

Participant characteristics were presented in the whole study population as well as in men and women separately as mean (SD; for normally distributed continuous data), as median (interquartile range; for nonnormally distributed data) or as proportion (for categorical variables).

We first performed multivariable-adjusted logistic regression analyses to investigate the associations between

self-reported hypo- and hyperthyroidism and anemia. Two models were constructed; a minimally adjusted model comprising age and sex, and a fully adjusted model additionally comprising natural log–transformed hs-CRP, smoking status, and alcohol intake. For sensitivity purposes, the dichotomous outcome of anemia was supplemented with analyses of Hb as a continuous outcome. Similar models were constructed as above but applied to multivariable-adjusted linear regression to assess the difference in Hb in g/dL.

Subsequently, we assessed the association of genetically influenced TSH and fT4 concentration, as instrumental variables, with anemia in the total study population. Since the previously found association, based on a large multicohort analysis, between thyroid function and anemia by Wopereis et al was U-shaped (12), the genetic risk scores were divided into equal-sized tertiles. With the middle group as reference, a multivariable-adjusted logistic regression model was constructed adjusted for age, sex, and the first 10 principal components to correct for possible population stratification. Additional analyses were performed with the lowest group as reference. Similar models were built for the genetic risk scores for deiodinase activity, again with the middle tertile as a reference group. To assess consistency, similar models were constructed to investigate the difference in Hb in g/dL between the highest and lowest tertile of genetic risk scores compared with the middle tertile using multivariable-adjusted linear regression.

To assess sex-specific associations, the main analyses were also performed stratified by sex and interaction analyses (on a multiplicative scale) were performed when sex differences were apparent by including an interaction term between sex and the exposure in the logistic regression model. In addition, summary-level data-based methods for MR were employed to assess whether unbalanced directional pleiotropy may have biased our analyses on genetically determined TSH and fT4 with anemia. The inverse variance–weighted (IVW) analysis provides a weighted mean estimate of the association of the individual genetic variants which assumes that none of the instruments were invalid (22). In addition, the weighted median estimator (WME), MR Egger regression, and MR pleiotropy residual sum and outlier (MR-PRESSO) analyses were employed, as they do take into account possible bias caused by directional pleiotropy based on different assumptions on the number of invalid instrumental variables (22, 23).

The results for the multivariable logistic regression of self-reported hypo- and hyperthyroidism are presented as the estimated odds ratio (OR) of having anemia for those who reported hypo- or hyperthyroidism compared with those without thyroid disease with the accompanying 95% CI. For the multivariable logistic regression of tertiles of genetic risk score, the OR and 95% CI of having anemia are given for the 33% with the highest and the lowest genetically determined TSH and fT4 compared with the middle third. For the multivariable linear regression analyses, we present the estimated difference in Hb concentration (expressed in g/dL) between the groups with accompanying 95% CI. All analyses and data visualization were performed in R version 3.6.1 (24) supplemented with the following packages: MRCIEU/TwoSampleMR (25), rondolab/MR-PRESSO (23), metafor (26), and ggplot2 (27).

Results

Participant Characteristics

Baseline characteristics of the UKB participants are displayed in Table 1. Of the 445 482 participants included in this study, 241 337 (54.2%) were women. The mean age ± SD was 56.8 ± 8.0 years, which was similar in men (57.0 ± 8.1) and women (56.6 ± 7.9). Men were more frequently current smokers (12.2%) than women (8.9%) and more likely to drink alcoholic beverages more than twice a week (53.1% of men, 38.1% of women). Among women, there was a higher prevalence of both self-reported clinical diagnosis of hypothyroidism (7.7%) and hyperthyroidism (1.2%) compared with the respective prevalence of 1.6% and 0.3% among men. In agreement with this, a higher use of T4 suppletion was reported in women (8.8%) than in men (1.9%), as was the reported use of T3 suppletion and antithyroid medication. Mean Hb level ± SD was 15.0 ± 1.0 g/dL for men and 13.5 ± 1.0 g/dL for women. A total of 18 717 (4.2%) of participants had anemia; 5907 (2.9%) were men and 12 810 (5.3%) women.

Associations Between Self-Reported Clinical Diagnosis of Hypo- and Hyperthyroidism With Anemia

Associations between self-reported clinical diagnosis of hypo- and hyperthyroidism and anemia are shown in Table 2. Self-reported clinical diagnosis of hypothyroidism was associated with higher risk of anemia (OR 1.12 [95% CI 1.05, 1.19]; P value 6.51×10^{-4}), independent of C-reactive protein, alcohol intake, and smoking. However, the effect estimates of these associations were stronger in men than in women (P value for interaction 9.48×10^{-15}). Although analyses for self-reported clinical diagnosis hyperthyroidism showed a similar direction of effect (OR 1.09), this observation was not supported by evidence from statistical testing (95% CI, 0.91; 1.30). Similar to the analyses on self-reported clinical diagnosis of hypothyroidism, the risk estimate was slightly higher in men than in women (P value for interaction 0.09).

Table 1. Participant characteristics at the baseline visit of the UK Biobank

	All (N = 445 482)	**Men** (N = 204 145)	**Women** (N = 241 337)
Age in years, mean ± SD	56.8 ± 8.0	57.0 ± 8.1	56.6 ± 7.9
Smoking currently, n (%)	46 412 (10.5%)	24 903 (12.2%)	21 509 (8.9%)
Alcohol intake > 2 times/week, n (%)	200 322 (45.0%)	108 334 (53.1%)	91 988 (38.1%)
hs-CRP, median (IQR)	1.33 (0.66-2.75)	1.28 (0.66-2.55)	1.37 (0.65-2.95)
GRS TSH, mean ± SD	3.11 ± 0.32	3.11 ± 0.32	3.11 ± 0.32
GRS fT4, mean ± SD	2.22 ± 0.21	2.22 ± 0.21	2.22 ± 0.21
Self-reported clinical diagnosis of thyroid disorder	21 860 (4.9%)	3236 (1.6%)	18 624 (7.7%)
Hypothyroidism, n (%)			
Hyperthyroidism, n (%)	3431 (0.8%)	627 (0.3%)	2804 (1.2%)
Thyroid hormone suppletion	123 (0.03%)	15 (0.01%)	108 (0.04%)
T3, n (%)			
T4, n (%)	25 021 (5.6%)	3914 (1.9%)	21 107 (8.8%)
Antithyroid medication, n (%)	383 (0.09%)	79 (0.04%)	304 (0.13%)
Hb, mean ± SD	14.19 ± 1.23	15.00 ± 1.02	13.51 ± 0.95
Anemia, n (%)	18 717 (4.2%)	5907 (2.9%)	12 810 (5.3%)

Anemia defined as hemoglobin < 13 g/dL for men and hemoglobin < 12 g/dL for women.
Abbreviations: BMI, body mass index; fT4, free thyroxine; GRS, genetic risk score; Hb, hemoglobin; IQR, interquartile range; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.

Results with Hb as a continuous outcome were directionally consistent for hypothyroidism (–0.02 g/dL [95% CI, –0.03; –0.01] *P* value 7.09×10^{-3}), with a larger effect estimate in men (–0.13 g/dL [95% CI, –0.17; –0.10]) than in women (–0.02 g/dL [95% CI, –0.04; –0.01]) (Supplementary Table 1 (28)). For hyperthyroidism, results were inconsistent with a self-reported clinical diagnosis of hyperthyroidism being associated with higher Hb in all (0.04 g/dL [95% CI, 0.01; 0.08] *P* value 0.03) and in women (0.05 g/dL [95% CI, 0.00; 0.08]), while with lower Hb in men (–0.03 g/dL [95% CI –0.13; 0.06]).

Genetically Influenced TSH and fT4 in Relation to the Risk of Anemia

For the MR analyses, TSH and fT4 levels were instrumented in weighted genetic risk scores based on effect estimates of individuals SNPs derived from previous genetic meta-analyses conducted in euthyroid European participants (16). In general, genetically influenced higher TSH was not associated with risk of anemia. Furthermore, neither individuals with lower genetically influenced TSH nor those with higher genetically influenced TSH (expressed as the lower and higher third of the TSH genetic risk score) had an increased risk of having anemia (compared with the middle tertile: OR for lowest tertile 0.98 [95% CI, 0.95-1.02]; highest tertile 1.02 [95% CI, 0.98-1.06]) (Table 3). In addition, genetically influenced fT4 above or below the reference group was not associated with anemia risk (compared with the middle tertile: OR for lowest tertile 1.00 [95% CI, 0.96-1.03]; highest tertile 0.99 [95% CI, 0.95-1.03]). Similar results were observed for men and women. When we used the group with the lowest TSH genetic risk scores as reference, individuals in the highest group had a higher risk of anemia (OR 1.04; 95% CI, 1.00-1.08), with particular evidence present in women (OR 1.05; 95% CI, 1.00-1.10). No evidence was found for fT4 and in men (Supplementary Table 2 (28)).

Sensitivity analyses on genetically influenced TSH or fT4 in relation to Hb did not yield any associations either (Supplementary Table 3 (28)). Although some associations between genetically influenced fT4 and Hb were observed in either men or women only, effect estimates were very small (men with fT4 below average –0.01 g/dL, 95% CI –0.02; 0.00; women with fT4 above average 0.01 g/dL, 95% CI 0.00; 0.02).

Sensitivity analyses using summary-level data-based methods for MR showed similar results, and the MR Egger intercepts and MR-PRESSO distortion tests did not indicate presence of severe unbalanced directional pleiotropy except possibly for fT4 and anemia (distortion test *P* = 0.04) (Supplementary Table 4, Supplementary Figures 1-4 (28)).

Genetic Variation in Deiodinases and Anemia

Individuals who had genetic variants in *DIO3OS* that were associated with fT4 above or below average had a slightly higher risk of anemia than those in the average group (OR lowest tertile 1.04 [95% CI, 1.00-1.08]; highest tertile 1.05 [95% CI, 1.02-1.10]) (Table 4). The individual SNPs in *DIO3OS* had similar effect estimates on risk of anemia, though the width of the confidence interval differed

Table 2. Associations between self-reported clinical diagnosis of hypothyroidism or hyperthyroidism and anemia compared with participants without a self-reported clinical diagnosis of thyroid dysfunction

	All			Men			Women		
	N exposed/ N unexposed	OR (95% CI)	*P* value	N exposed/ N unexposed	OR (95% CI)	*P* value	N exposed/ N unexposed	OR (95% CI)	*P* value
Hypothyroidism									
Model 1	20 980/ 421 071	1.18 (1.11; 1.25)	1.31×10^{-7}	3103/200 415	1.83 (1.56; 2.13)	1.69×10^{-14}	17 877/220 656	1.17 (1.09; 1.25)	4.30×10^{-6}
Model 2	19 959/401 260	1.12 (1.05; 1.19)	6.51×10^{-4}	2942/190 877	1.71 (1.45; 2.00)	4.46×10^{-11}	17 017/210 383	1.12 (1.05; 1.20)	1.01×10^{-3}
Hyperthyroidism									
Model 1	2551/421 071	1.14 (0.95; 1.35)	0.151	494/200 415	1.49 (0.94; 2.23)	0.068	2057/220 656	1.13 (0.93; 1.37)	0.195
Model 2	2429/401 260	1.09 (0.91; 1.30)	0.340	466/190 877	1.54 (0.97; 2.32)	0.050	1963/210 383	1.09 (0.89; 1.32)	0.413

All models were adjusted for age; analyses in men and women combined were additionally adjusted for sex; Model 2 was additionally adjusted for CRP, current smoking, and alcohol intake more than twice a week.
N exposed refers to the number of individuals included in the analysis exposed to thyroid disease, the N unexposed refers to the number of included individuals without thyroid disease. Hypothyroidism and hyperthyroidism were self-reported, anemia was defined as hemoglobin < 13 g/dL for men and hemoglobin < 12 g/dL for women.
Abbreviation: OR, odds ratio.

(Supplementary Table 5 (28)). No associations were observed with genetic variation in *DIO1* and *DIO2*. In sensitivity analyses, genetic variation in *DIO1*, *DIO2,* and *DIO3OS* was not associated with Hb (Supplementary Table 6 (28)).

Discussion

In this study, multivariable-adjusted and Mendelian randomization analyses were performed in a large population of individuals of European descent to examine a possible relationship between thyroid function and anemia. We found that individuals in our study population with a self-reported clinical diagnosis of hypothyroidism had a higher risk of anemia compared with those without thyroid diseases, especially in men. However, we observed no consistent evidence favoring an association between genetically influenced variation in circulating TSH and fT4 levels and anemia using multiple statistical methodologies and approaches, suggesting the observed association between the self-reported clinical diagnosis of hypo- or hyperthyroidism with anemia cannot be extrapolated to variation in thyroid function within the euthyroid range. However, in explorative analyses specifically looking at variation in genes encoding deiodinases, a novel significant U-shaped association between variants in the *DIO3OS* gene and risk of anemia was found.

A higher risk of anemia in relation to thyroid diseases has been described previously. In the EPIC-Norfolk cohort, overt hypo- and hyperthyroidism were associated with higher risk of anemia while subclinical thyroid dysfunction was not (13, 14). In an individual participant meta-analysis of 16 cohorts, hypo- and hyperthyroidism were associated with higher prevalence of anemia, and the risk was higher for those with overt thyroid dysfunction than in subclinical disease (12). In the present study, we observed similar results through self-reported data on hypothyroidism and showed that these associations are independent of (low-grade) systemic inflammation and related lifestyle factors. Adjustment for these factors did not explain the observed difference in association between men and women; it is currently unclear which mechanisms/factors contribute to the higher thyroid dysfunction–associated anemia risk in men. Speculatively, the difference in strength of the association between thyroid dysfunction and anemia could be due to differential misclassification bias or competing risk. Since men in the UK consult their GP less frequently than women with similar conditions (29), it could be that men only consult a clinician with severe thyroid dysfunction while women are more likely to consult with milder thyroid dysfunction, leading to a relatively more severely affected population of men with a self-reported diagnosis than for

Table 3. Genetically influenced thyroid status of TSH and fT4 and anemia in the UK Biobank population[a]

	All		Men		Women	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Genetically influenced TSH						
Lowest tertile	0.98 (0.95; 1.02)	0.427	1.03 (0.96; 1.10)	0.417	0.96 (0.92; 1.01)	0.125
Middle tertile	Reference		Reference		Reference	
Highest tertile	1.02 (0.98; 1.06)	0.247	1.04 (0.97; 1.11)	0.243	1.01 (0.97; 1.06)	0.534
Genetically influenced fT4						
Lowest tertile	1.00 (0.96; 1.03)	0.835	1.03 (0.96; 1.10)	0.418	0.98 (0.94; 1.03)	0.434
Middle tertile	Reference		Reference		Reference	
Highest tertile	0.99 (0.95; 1.03)	0.649	0.99 (0.92; 1.06)	0.711	0.99 (0.95; 1.04)	0.765

All analyses were adjusted for age and the first 10 principal components to correct for possible population stratification; analyses in men and women combined were additionally adjusted for sex.
Abbreviations: fT4, free thyroxine; OR, odds ratio; TSH, thyrotropin (thyroid-stimulating hormone).
[a]Anemia defined as hemoglobin < 13 g/dL for men and hemoglobin < 12 g/dL for women

Table 4. Genetic variation in deiodinase genes and anemia in the UK Biobank population[a]

	DIO1		*DIO2*		*DIO3OS*	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Lowest tertile	0.98 (0.95; 1.02)	0.395	0.98 (0.95; 1.02)	0.365	1.04 (1.00; 1.08)	0.070
Middle tertile	Reference		Reference		Reference	
Highest tertile	0.98 (0.94; 1.02)	0.260	1.02 (0.97; 1.07)	0.459	1.05 (1.02; 1.10)	0.006

All analyses were adjusted for age; analyses in men and women combined were additionally adjusted for sex.
Abbreviation: OR, odds ratio.
[a]Anemia defined as hemoglobin < 13 g/dL for men and hemoglobin < 12 g/dL for women

the women. Moreover, women generally have a higher risk of anemia than men, due to other causes of anemia such as blood loss during menstruation and nutritional deficiencies of iron and other crucial micronutrients (1, 30). However, this all remains speculation; exploration of these contributing factors for these sex differences was beyond the scope of the present study. Further research is required to address these sex-specific mechanisms.

Although this is the first study using Mendelian randomization to study genetically influenced TSH and fT4 levels in relation to anemia, various studies did assess this association using other research designs. Two previous cross-sectional studies also did not observe an association between circulating TSH and Hb levels in euthyroid populations, although both did find an association between low fT4 and lower Hb (31, 32). In our analyses, individuals with lower genetically influenced fT4 level also had lower Hb, but the effect estimates were very small. No association was found between genetically influenced fT4 and risk of anemia.

Various explanations are possible for the observed discordance between the results from the multivariable-adjusted analyses and the MR analyses. On the one hand, it is possible that the observed association between hypothyroidism and anemia is due to confounding and selection bias of people having their thyroid function checked. For instance, fatigue and tiredness are common reasons to have thyroid function checked. On the other hand, individuals with a diagnosis of hypo- or hyperthyroidism are usually treated, although thyroid hormone use in older people has been associated with a high frequency of thyroid hormone over- or under-replacement (33, 34). Consequently, self-reported thyroid dysfunction is not analogous to real (biochemical) deficit or excess of thyroid hormones, and the presence of over- and undertreatment complicate interpretation. It is likely that within the group who have a diagnosis of hypo- or hyperthyroidism more individuals have a biochemical thyroid function outside the reference range than among those without such diagnosis, due to either persistent disease or over- or undertreatment. Another possibility is that people with a diagnosis of hypothyroidism who are treated with levothyroxine have a lower fT3/fT4 ratio when TSH is normalized, which may have contributed to or driven the observed association. Unfortunately, the potential causal association of fT3 or fT3/fT4 ratio and anemia could not be assessed by MR, as instruments for

fT3 and fT3/fT4 ratio were not available. For circulating levels of TSH and fT4 we did have genetic instruments, although only for variation within the reference range. Therefore, the MR analyses address a subtly different research question than the multivariable-adjusted regression analyses, namely the association between variation in circulating TSH and fT4 within the reference range and the risk of anemia. Potentially erythropoiesis is only hampered by true deficit or excess thyroid hormone beyond buffering capacity, which would not arise within the reference range in individuals without defects in thyroid hormone receptors or other signaling pathway constituents. In summary, differences in findings could be due to selection bias and residual confounding in the multivariable-adjusted regression analyses, or due to differences in exposures assessed by the different methods with different potential downstream effects.

The production of sufficient numbers of differentiated red blood cells from hematopoietic progenitor cells is dependent on a delicate balance between proliferation and differentiation of progenitor cells. Results from studies on ex vivo cultures from RTHα patients suggest that reduced intracellular thyroid hormone action results in a disturbed balance between proliferation and differentiation of erythrocytic progenitors, which may contribute to anemia (11). To the best of our knowledge, this is the first study to report a significant U-shaped association between genetic variants in the *DIO3OS* gene and anemia risk. Although the exact biological function of *DIO3OS* remains thus far unclear, it is hypothesized that it may be involved in *DIO3* expression levels (35). Temporary induction of D3 induces stem cell proliferation (36), which is also required in erythropoiesis. However, no literature specifically on the role of D3 in erythrocytes or erythropoiesis was found.

Different from the thyroid hormone levels in blood, thyroid hormone deactivation by D3 might affect the risk of anemia. These findings may appear contradictory at first sight, although we hypothesize that they indicate a crucial role for local regulation of thyroid hormone availability in the development of disease. Thyroid signaling can be customized at the cellular level through regulation of thyroid hormone transporters, deiodinases, and nuclear thyroid hormone receptors among others (6). The intracellular exposure to T3 is thereby to some extent independent of centrally regulated TSH and fT4, which is especially important in orchestrating proliferation and differentiation of stem cells and progenitor cells (7). Because of this local regulation, processes which are sensitive to thyroid hormone levels might be protected from subtle variations in circulating levels. However, when circulating thyroid hormone levels are extremely high or extremely low, these local compensatory mechanisms might no longer suffice, as illustrated previously by Bassett et al (37). In line with this, when the machinery for regulation does not work optimally (ie, because of a polymorphism in *DIO2*), local regulation is impaired and cells become more sensitive to changes in circulating levels of thyroid hormones (38). Based on our results, we now hypothesize that functional genetic variants in *DIO3OS* result in a higher risk of anemia driven by a suboptimal intracellular regulation of thyroid hormone inactivation, although this hypothesis requires additional work and functional validation.

The current study has a few noteworthy strengths. First of all, the large sample size of the UK Biobank results in precise effect estimation and allows for stratified analyses even with rare exposures such as hyperthyroidism. Secondly, the combination of different approaches shed light on multiple facets of thyroid function in relation to development of anemia. Together, these new insights add to the etiological understanding of the relationship between thyroid function and anemia. There are also some limitations to the present study. There may be a degree of selection bias, as individuals who responded to the UK Biobank invitation and attended the assessments are healthier than the general population (18). In addition, symptoms of anemia, such as fatigue and tiredness, may have been common reasons for people to have the thyroid function checked. Furthermore, the clinical diagnosis of hypo- and hyperthyroidism were based on self-report; although the interview was conducted by a trained research nurse, some misclassification cannot be excluded. Moreover, observed associations of self-reported hypo- or hyperthyroidism with anemia may have been suffered from attenuation due to normalization caused by treatment. However, thyroid hormone use in older adults has been associated with a high frequency of thyroid hormone over- or under-replacement (33, 34). Consequently, individuals with self-reported thyroid dysfunction are more likely than those without such diagnosis to be exposed to (biochemical) deficit or excess of thyroid hormones. Unfortunately, we were unable to prove this in the present study due to lack of data, so this therefore remains a hypothesis. Anemia was measured objectively in blood samples taken at the visit; however, variation in blood count caused by laboratory drift cannot be ruled out as hematological assays were performed throughout the recruitment period (39). However, this has likely not played a role in the analyses we present in our studies given that this increased variation is most likely caused independently from thyroid function. Finally, current analyses were restricted to participants of European ancestry, limiting the generalizability of results to other ancestral groups.

In summary, among individuals of European ancestry participating in UK Biobank, hypothyroidism was associated with a higher risk of anemia independent of inflammation

and lifestyle. Genetically determined variation in circulating levels of TSH and fT4 within the reference range is not associated with anemia, although intracellular regulation of thyroid hormones via *DIO3OS* might play a role in development of anemia. Further studies are required to unravel the molecular mechanisms underlying the complex relationship between thyroid hormones and anemia risk.

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References

1. Kassebaum NJ; GBD 2013 Anemia Collaborators. The global burden of Anemia. *Hematol Oncol Clin North Am*. 2016;**30**(2):247-308.
2. World Health Organization. *Nutritional Anaemias: Report of A WHO Scientific Group*. Geneva, Switzerland: World Health Organization; 1968.
3. Tettamanti M, Lucca U, Gandini F, et al. Prevalence, incidence and types of mild anemia in the elderly: the "Health and Anemia" population-based study. *Haematologica*. 2010;**95**(11):1849-1856.
4. Girelli D, Marchi G, Camaschella C. Anemia in the elderly. *Hemasphere*. 2018;**2**(3):e40.
5. Szczepanek-Parulska E, Hernik A, Ruchała M. Anemia in thyroid diseases. *Pol Arch Intern Med*. 2017;**127**(5):352-360.
6. Bianco AC, Dumitrescu A, Gereben B, et al. Paradigms of dynamic control of thyroid hormone signaling. *Endocr Rev*. 2019;**40**(4):1000-1047.
7. Luongo C, Dentice M, Salvatore D. Deiodinases and their intricate role in thyroid hormone homeostasis. *Nat Rev Endocrinol*. 2019;**15**(8):479-488.
8. Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. *Lancet*. 2017;**390**(10101):1550-1562.
9. De Leo S, Lee SY, Braverman LE. Hyperthyroidism. *Lancet*. 2016;**388**(10047):906-918.
10. Park S, Han CR, Park JW, et al. Defective erythropoiesis caused by mutations of the thyroid hormone receptor α gene. *PLoS Genet*. 2017;**13**(9):e1006991.
11. van Gucht ALM, Meima ME, Moran C, et al. Anemia in patients with resistance to thyroid hormone α: a role for thyroid hormone receptor α in human erythropoiesis. *J Clin Endocrinol Metab*. 2017;**102**(9):3517-3525.
12. Wopereis DM, Du Puy RS, van Heemst D, et al; Thyroid Studies Collaboration. The relation between thyroid function and anemia: a pooled analysis of individual participant data. *J Clin Endocrinol Metab*. 2018;**103**(10):3658-3667.
13. M'Rabet-Bensalah K, Aubert CE, Coslovsky M, et al. Thyroid dysfunction and anaemia in a large population-based study. *Clin Endocrinol (Oxf)*. 2016;**84**(4):627-631.
14. Floriani C, Feller M, Aubert CE, et al. Thyroid dysfunction and anemia: a prospective cohort study and a systematic review. *Thyroid*. 2018;**28**(5):575-582.
15. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;**362**:k601.
16. Teumer A, Chaker L, Groeneweg S, et al; Lifelines Cohort Study. Genome-wide analyses identify a role for SLC17A4 and AADAT in thyroid hormone regulation. *Nat Commun*. 2018;**9**(1):4455.
17. 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**(3):e1001779.
18. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol*. 2017;**186**(9):1026-1034.
19. Marouli E, Kus A, Del Greco MF, et al. Thyroid function affects the risk of stroke via atrial fibrillation: a Mendelian randomization study. *J Clin Endocrinol Metab*. 2020;**105**(8):2634-2641.
20. de Vries EM, Fliers E, Boelen A. The molecular basis of the non-thyroidal illness syndrome. *J Endocrinol*. 2015;**225**(3):R67-R81.
21. Fraenkel PG. Anemia of inflammation: a review. *Med Clin North Am*. 2017;**101**(2):285-296.
22. Bowden J, Holmes MV. Meta-analysis and Mendelian randomization: a review. *Res Synth Methods*. 2019;**10**(4):486-496.
23. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;**50**(5):693-698.
24. R Core Team. *R: A Language and Environment for Statistical Computing*. 3.6.1 ed. Vienna, Austria: R Foundation for Statistical Computing; 2019.
25. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife*. 2018;**7**:e34408.
26. Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw*. 2010;**36**(3):48.
27. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag; 2009.
28. van Vliet NA, Kamphuis AEP, den Elzen WP, et al. Thyroid function and risk of anemia: a multivariable-adjusted and Mendelian Randomization analysis in the UK Biobank: supplemental materials. *figshare*. Updated September 5, 2021. https://figshare.com/s/8022cc1fad4e48b50c13
29. Wang Y, Hunt K, Nazareth I, Freemantle N, Petersen I. Do men consult less than women? An analysis of routinely collected UK general practice data. *BMJ Open*. 2013;**3**(8):e003320.

30. Derbyshire E. Micronutrient intakes of British adults across mid-life: a secondary analysis of the UK national diet and nutrition survey. *Front Nutr.* 2018;**5**:55. doi:10.3389/fnut.2018.00055
31. Schindhelm RK, ten Boekel E, Heima NE, van Schoor NM, Simsek S. Thyroid hormones and erythrocyte indices in a cohort of euthyroid older subjects. *Eur J Intern Med.* 2013;**24**(3):241-244.
32. Kim M, Kim BH, Lee H, et al. Association between serum free thyroxine and anemia in euthyroid adults: a nationwide study. *Endocrinol Metab (Seoul)*. 2020;**35**(1):106-114.
33. Somwaru LL, Arnold AM, Joshi N, Fried LP, Cappola AR. High frequency of and factors associated with thyroid hormone over-replacement and under-replacement in men and women aged 65 and over. *J Clin Endocrinol Metab*. 2009;**94**(4):1342-1345.
34. Taylor PN, Iqbal A, Minassian C, et al. Falling threshold for treatment of borderline elevated thyrotropin levels-balancing benefits and risks: evidence from a large community-based study. *JAMA Intern Med*. 2014;**174**(1):32-39.
35. Kuś A, Chaker L, Teumer A, Peeters RP, Medici M. The genetic basis of thyroid function: novel findings and new approaches. *J Clin Endocrinol Metab*. 2020;**105**(6):1707-1721.
36. Salvatore D. Deiodinases and stem cells: an intimate relationship. *J Endocrinol Invest*. 2018;**41**(1):59-66.
37. Bassett JH, Williams GR. Role of thyroid hormones in skeletal development and bone maintenance. *Endocr Rev.* 2016;**37**(2):135-187.
38. Jo S, Fonseca TL, Bocco BMLC, et al. Type 2 deiodinase polymorphism causes ER stress and hypothyroidism in the brain. *J Clin Invest*. 2019;**129**(1):230-245.
39. Elliott P, Peakman TC; UK Biobank. The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. *Int J Epidemiol.* 2008;**37**(2):234-244.