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Thyroid function and risk of atrial fibrillation and kidney disease

- A Mendelian Randomization Study

by

Christina Ellervik

A Dissertation Submitted to the Faculty of Harvard Medical School

in Partial Fulfillment of the Requirements for

the Degree of Master of Medical Sciences in Clinical Investigation (MMSCI)

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Area of Concentration: Genetic epidemiology/endocrinology

Project Advisor Dr. Sagar U Nigwekar and Finnian R. McCausland

I have reviewed this thesis. It represents work done by the author under my guidance/supervision.

Primary Mentor: Dan I. Chasman

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Overview of the thesis projects

Often, the observational epidemiological study design has the limitation of being confounded, prone to selection bias, and prone to reverse causation. The Mendelian randomization (MR) design may overcome these problems as it mimicks a randomized double-blind clinical trial design(1, 2). The MR design in human genetics thus relies on the random assortment of alleles at conception as it ensures random distribution of confounding factors and thereby this approach circumvents reverse causation. In the MR design, one or several genetic polymorphisms are used as proxies for a lifelong exposure to an intermediate trait.

In previous observational studies, hypothyroidism(3-14) and increased thyroid stimulating hormone within the reference range (3, 15-21) have been associated with reduced kidney function. Furthermore, in previous observational studies, hyperthyroidism and increased free thyroxine (fT4) have been associated with increased risk of atrial fibrillation(22-25). However, whether these observations are causal are unknown. Therefore, in this thesis, we use a MR design to assess causality for these observations. We use the Women's Genome Health Study (WGHS)(26) and data from two consortia, the Atrial Fibrillation Genetics Consortium (AFGen)(27) and the Chronic Kidney Disease Genetics Consortium (CKGen)(28). We use information from previous GWAS studies on thyroid function(29-32), kidney disease(28), and atrial fibrillation(27).

Project 1: Hypothyroidism and kidney function – A Mendelian Randomization study

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Abstract

Background: Thyroid and kidney dysfunction correlate in observational studies but causality and directionality are uncertain.

Methods: In Women's Genome Health Study (WGHS), we investigated observational associations between thyroid function (thyroid stimulating hormone (TSH), free T4 hormone (fT4), and fT3, N=3,336) and estimated glomerular filtration rate (eGFR/N=23,294). Common genetic SNPs for hypothyroidism, TSH and fT4 concentrations within the reference range, thyroperoxidase antibody (TPOAb), and kidney function were used in bidirectional Mendelian Randomization (MR) in the WGHS. The genetic effect of thyroid function on kidney function was also explored in genetic association summary statistics from the Chronic Kidney Disease Genetics Consortium (CKDGen) for eGFR from creatinine ($eGFR_{crea}$/N=133,413) and cystatin-C ($eGFR_{cys}$/N=32,824), chronic kidney disease (CKD/N=117,165), and urinary albumin creatinine ratio (UACR/N=54,450).

Results: In the WGHS, hypothyroidism was associated with decreased $eGFR_{crea}$ observationally [beta (SE); -0.026(0.009) ln(mL/min/1.73 m2);p=0.00561] and genetically [-0.012(0.007); p=0.084)] using 4 SNPs in inverse variance weighted analysis (IVW). In the larger sample from CKDGen, genetically predicted hypothyroidism was associated with significantly decreased $eGFR_{crea}$ [-0.0093(0.0026),p=0.00036] by IVW which was robust to MR sensitivity analysis and primarily driven by autoimmune loci; however, there was no association between genetically predicted hypothyroidism and $eGFR_{cys}$, CKD or UACR. Genetically predicted increased TSH, fT4, and TPOAb in WGHS and CKDGen were not associated with decreased $eGFR_{crea}$, $eGFR_{cys}$, CKD or UACR.

Conclusion: This bidirectional MR study found evidence for causality in correlations between hypothyroidism and decreased kidney function consistent with an autoimmune mechanism, but not between thyroid function in the normal range and kidney function.

Significance statement:

1) The direction and causality between thyroid function and kidney function is disputed

2) Genetically predicted hypothyroidism is associated with a decreased kidney function

3) The pathway is likely autoimmune mediated

Introduction

Subclinical hypothyroidism is characterized by high concentration of thyroid stimulating hormone (TSH) but with thyroid hormones concentrations within the reference range. Primary overt hypothyroidism, characterized by high concentration of thyroid stimulating hormone (TSH) and low concentration of thyroid hormones, is most often an autoimmune disease in adults (33). Overt and subclinical hypothyroidism are associated with increased creatinine, decreased estimated glomerular filtration rate (eGFR), chronic kidney disease (CKD), and increased urinary albumin creatinine ratio (UACR)(3-14). The co-occurrence of autoimmune hypothyroidism and kidney disease is considered rare and is based on renal biopsy findings in patients with hypothyroidism described in scattered case reports or case series(34-37). The various glomerular diseases described in hypothyroidism include membranous glomerulonephritis, focal segmental glomerulosclerosis, IgA nephropathy, chronic glomerulonephritis, and minimal change disease(34-37). Several pathological mechanisms could likely explain these observations including glomerular deposition of immune complexes of thyroglobulin and autoantibodies(37), a decreased cardiac output leading to decreased renal blood flow with a resulting decreased GFR(38), or a shared underlying etiology as hypothyroidism often coexist with other autoimmune diseases with renal impairment, such as amyloidosis, rheumatoid arthritis, and systemic lupus erythematosus (SLE)(39). However, kidney disorders may also be related to thyroid dysfunction due to leakage of proteins into the urine (including TSH, T4, and binding proteins) or non-thyroidal illness(40-43).

TSH is the principal regulator of thyroid hormone (T3 and T4) synthesis and secretion and is regulated by negative feedback. Thyroid hormones within the reference range may affect kidney function through various mechanisms, including a direct effect on glomerular and tubular functions and an indirect pre-renal effect through cardiovascular hemodynamics and renal blood flow(40). Increased TSH within the reference range is associated with reduced eGFR (3, 15-21).

But whether increased thyroxine (T4) is associated with a decline or increase in kidney function is debated (5, 6, 15, 17, 21, 44, 45), as is the possibility that increased TSH is linked to chronic kidney disease in euthyroid individuals (3, 15-19, 45).

Thus, the direction and causality between thyroid function and kidney function cannot fully be addressed by observational studies, due to confounding and reverse causation. Observational designs or small randomized trials show that thyroid hormone replacement therapy improves kidney function in patients with subclinical or overt hypothyroidism (46-50). But no large-scale, long-term randomized trial has been undertaken to investigate how hormone replacement therapy influences kidney function in patients with hypothyroidism. Thus, an alternative approach to understanding causality along the thyroid-kidney axis is to employ a Mendelian Randomization (MR) design, using genetic SNPs as proxies to indirectly infer causality for thyroid function (51). The assumption in MR is that a random assortment of alleles at conception ensures a balanced distribution of confounders across the genotypes, thereby circumventing reverse causation and mimicking a randomized trial. Employed in a bidirectional design, MR can be used to evaluate the direction of potential causality between thyroid and kidney function.

Here we have used bidirectional MR, to meet our primary aim, namely, to assess the evidence for potential causality and direction between thyroid function and kidney function. We used genetically different thyroid function instruments using common GWAS SNPs for hypothyroidism(32, 52), increased TSH within the reference range(29, 31), increased fT4 within the reference range(29, 31), and increased antibodies against thyroid peroxidase (TPOAb) (30, 53), in order to predict kidney function ($eGFR_{crea}$, $eGFR_{cys}$, CKD(28), and UACR(54)) in the Women's Genome Health Study (WGHS) and through published summary statistics from the Chronic Kidney Disease Genetics Consortium (CKGen). In addition, in the WGHS, we used GWAS-identified

instruments for kidney function as instruments for thyroid function to predict kidney function, and thereby investigate reverse causality (Figure 1).

Methods

Women's Genome Health Study (WGHS)

The WGHS is a prospective cohort of North American women, 45 years or older, and free of cardiovascular disease and cancer at the time of enrolment for whom whole genome genotype information is available(26). The WGHS includes 23,294 women with self-reported European ancestry that was verified by genetic analysis. Participating women gave written informed consent at enrolment. The study was approved by the Institutional Review Board at the Brigham and Women's Hospital (Boston, MA).

Although, information about thyroid disease or thyroid medication at baseline is not available in the WGHS, measures of thyroid function were obtained in a random subset of WGHS participants from biobanked plasma stored in vapor-phase liquid nitrogen (170°C) at the time of enrolment(55). TSH (mIU/ml, N=3,336), free T3 (fT3) (ng/dL, N=3,335), and free T4 (fT4) (ng/dL, N=3,335) were measured using the Roche Cobas e601 analyzer with coefficients of variation (CVs) of ≤3.3%, ≤4.6%, and ≤8.4%, respectively(55) at Atherotech Diagnostics Laboratory (Birmingham, AL). Eligible categories based on the Roche Cobas assay recommendations were defined as follows: euthyroid (TSH, 0.27 to 4.2 mIU/L; fT4, 0.93 to 1.7 ng/dL; N=2,256), subclinical and overt hypothyroidism combined (TSH> 4.2 mIU/L; fT4 ≤1.7 ng/dL; N=678)(55).

Serum creatinine (N=23,186) was measured on baseline blood samples by a rate-blanked method based on the Jaffe reaction, using reagents from Roche Diagnostics the reproducibility of the assay was 3.67% and 1.60% at concentrations of 1.17 and 6.40 mg/dL, respectively (26). Values of $eGFR_{crea}$ were calculated from serum creatinine, using the equation for the Modification of Diet in Renal Disease (MDRD) Study, applied to CKD ($eGFR_{crea}$ <60 ml/min/1.73m2)(28). $eGFR_{crea}$ was age- and gender-adjusted using residuals, and then natural log transformed(28).

Genotype data in the WGHS have been described(26). Briefly, whole genome genotypes were collected with Illumina HumanHap300 Duo "Plus" array. Genotypes for additional single nucleotide polymorphisms (SNPs) were determined by imputation to the 1000 genomes cosmopolitan reference panel from phase I, v. 3 (March 2012), using MACH software, version 1.0.16 (Center for Statistical Genetics, University of Michigan).

Genetic instruments for thyroid function

Common genetic SNPs with minor allele frequency (MAF)>1% have been identified in genome-wide association studies (GWAS) among Europeans for TSH (N=24) and fT4 (N=5) concentrations in the reference range(29, 31), for TPOAb concentration (N=5)(30, 53), for hypothyroidism (N=5)(32, 52), for kidney function and CKD (N=53)(28), and for UACR (N=1) (54) (**Supplementary Figure 1, Supplementary Table 1**). The SNPs for thyroid function only show very little overlap and therefore highlight different aspects of thyroid function in the analyses(29-32, 52, 53).

Genetic instruments for kidney function

Genetic instruments for kidney function were identified in the GWAS conducted by the Chronic Kidney Disease Genetics consortium (CKDGen). The CKDGen GWAS comprised 133,413 individuals across 48 studies for $eGFR_{crea}$ (28), 117,165 across 43 studies for CKD (28), 32,824 across 16 studies for $eGFR_{cys}$ (28), and 54,450 across 20 studies for UACR (54). In all studies, serum creatinine was calibrated to the US nationally representative NHANES data, to account for between-laboratory variation(28). Values of $eGFR_{crea}$ were calculated from serum creatinine, using the equation for the Modification of Diet in Renal Disease (MDRD) Study, applied to CKD

($eGFR_{crea}$ <60 ml/min/1.73m2)(28). $eGFR_{crea}$ was age- and gender-adjusted using residuals, and then natural log transformed(28). $eGFR_{cys}$ was estimated as 76.7*(serum cystatin C)$^{-1.19}$ (28). The UACR was calculated as urinary albumin/urinary creatinine (mg/g) to account for differences in urine concentration(54).

Statistics

In WGHS, we standardized TSH and fT4 to standard deviation (SD) units as the published effect sizes for the TSH SNPs are standardized, so they represent the estimated phenotypic change in SD units, per each copy of the effect allele (29, 31). Observational associations of TSH, fT4, fT3, and fT3/fT4-ratio with $eGFR_{crea}$ and CKD were investigated at a cross-sectional level using linear regression for continuous outcomes and logistic regression for binary outcomes. Analyses were adjusted for age at baseline (years), geographical location, systolic blood pressure (mm Hg), HbA1c (%), smoking (yes/no), total cholesterol (mg/dL), and BMI (kg/m^2). The statistical program R was used for WGHS analyses (v. 3.1.2).

Assumptions of MR are: a) the genotype is associated with the intermediary phenotype; b) no residual confounding exist between the genotype and the outcome; and c) the pathway from genotype to outcome passes only through the intermediary phenotype(51). Assumption a) is proven by the GWAS studies. Assumption b) cannot be proven, but in WGHS we tested known confounders across the genotypes. And assumption c) cannot be proven, but prior evidence can be used to support the unidirectionality of the pathway; nonetheless, pleiotropic effects may violate this assumption(51).

For MR analyses in the WGHS, instrument-exposure and instrument-outcome associations were estimated by linear regression for continuous measures of thyroid function (TSH,

fT4) and kidney function ($eGFR_{crea}$) while logistic regression was used for the binary measures of hypothyroidism and CKD (i.e. $eGFR_{crea}$ < 60 mL/min/1.73 m^2) categories, adjusting for age at baseline, geographical location and the first four principal components of population substrata for both types of models (**Figure 1)**. Previous GWAS results for TSH and fT4 were based on euthyroid individuals (29, 31). Therefore, in WGHS analyses involving TSH and fT4, we restricted the sample to euthyroid individuals (i.e., 0.27>=TSH<=4.2 mIU/l; N=2,256). For analyses of hypothyroidism (overt and subclinical combined), a subset of 2,474 euthyroid individuals was compared to 678 individuals with elevated TSH (>4.2mIU/l) and fT4<=1.7 ng/dL (the upper reference interval).

MR was conducted with the R packages MRInstruments and TwoSampleMR using R (v. 3.3.2)(56). We used two-sample MR analysis to determine the instrument-exposure for thyroid function and the instrument-outcome for kidney function (**Figure 1**) based on GWAS summary statistics from non-overlapping datasets(56) for hypothyroidism, TSH, fT4, and TPOAb ($\beta_{thyroid\text{-}SNP_thyroid\ function}$) (29-32, 52, 53) and for kidney function measures $eGFR_{crea}$, $eGFR_{cys}$, CKD, and UACR from CKDGen(28, 54).

For beta coefficients and standard errors for the inverse-variance weighted (IVW) MR analyses, we performed regression of the a) $\beta_{thyroid\text{-}SNP_kidney\ function}$ on the $\beta_{thyroid\text{-}SNP_thyroid\ function}$ in the forward direction (**Figure 1)**; and b) $\beta_{kidney\text{-}SNP_thyroid\ function}$ on the $\beta_{kidney\text{-}SNP_kidney\ function}$ in the reverse direction (**Figure 1**). The IVW method constrains the intercept of the regressions to the origin and assumes that all genetic SNPs are valid instruments (57). To assess if potential unmeasured pleiotropy or other unknown violations of MR assumptions may have influenced our findings, sensitivity analyses were performed using the MR Egger regression and weighted median (WM) methods. These methods have been shown to be robust to potential horizontal pleiotropic bias arising from inclusion of invalid instruments (57). MR Egger regression assumes that causal effects

of the genetic instruments are related to instrument strength but that pleiotropic effects of genetic SNPs are independent of instrumental strength. Under this assumption, removing the constraint of the regression line's intercept to the origin uncouples the potential causal and pleiotropic effects, although with a loss of power for the former. The weighted median assumes that 'instruments' representing more than 50% of the weight are valid IVs (57). We also performed sensitivity analyses using "leave one out" forest plots to account for exaggerated influence of individual SNPs.

Results

In the WGHS, 678 (20.3%) of 3,336 women with thyroid measures and European ancestry had hypothyroidism and 2,474 were euthyroid (**Supplementary Table 2**), while 2,124 (9.2%) of 23,294 women with European ancestry had CKD. Compared to euthyroid women, hypothyroid women were older, had higher systolic blood pressure, and were less often smokers. The majority of the genetic variants were not associated with the confounders, but rs925489 for hypothyroidism in *FOXE1* (Forkhead Box E1) was associated with younger age at baseline (Bonferroni corrected p=0.032) (**Supplementary Table 3**).

Bidirectional observational associations between thyroid function and kidney function in WGHS

Hypothyroidism was associated with a decreased $eGFR_{crea}$ [beta (SE); -0.026(0.009) ln(mL/min/1.73 m2);p=0.00561] but not with CKD (**Table 1**). Among all WGHS participants with TSH measures, increased TSH was also associated with a decreased $eGFR_{crea}$. In euthyroid participants, TSH or fT4 were not associated with $eGFR_{crea}$ or CKD. Increased $eGFR_{crea}$ was associated a decreased odds ratio for hypothyroidism [OR(95%CI); 0.55(0.36-0.85)], and with a decrease in TSH (**Table 1**). CKD was overall not associated with TSH, fT4 or hypothyroidism.

Bidirectional one-sample MR associations between thyroid function and kidney function in WGHS

We identified 4 SNPs for hypothyroidism, 23 SNPs for TSH, and 5 SNPs for fT4; the SNP-phenotype and SNP-outcome results are shown in **Supplementary Table 4**. Genetically predicted hypothyroidism was associated with a decreased $eGFR_{crea}$ [beta (SE); -0.012(0.007;p=0.084)] using 4 SNPs in the IVW analysis (**Table 2**); this effect estimate was not significantly different from the observational estimate in the WGHS (p=0.20). Genetically predicted increased TSH (per SD(mIU/L)) was also associated with a decreased $eGFR_{crea}$ [beta (SE); -0.013 (0.007)

ln(mL/min/1.73 m2);p=0.074] using 23 TSH SNPs in IVW analysis (**Table 2**). Genetically predicted hypothyroidism, TSH, or fT4 were not associated with CKD (**Table 2**).

Forty-four SNPs were identified for $eGFR_{crea}$, but we used only 40 of these, as 4 of them were palindromic with allele frequency near 0.5 and ambiguous coding; four SNPs were identified for CKD. The SNP-phenotype and SNP-outcome results are shown in **Supplementary Table 5.** Overall, neither genetically predicted $eGFR_{crea}$ nor CKD was associated with thyroid function in the reverse direction (**Table 2**).

Unidirectional two-sample MR associations between thyroid function and kidney function in the CKDGen consortium

Five SNPs for hypothyroidism, 17 SNPs for TSH, 4 SNPs for fT4, and 5 SNPs for TPOAb were represented in the CKDGen summary statistics for kidney function. The SNP-phenotype and SNP-outcome results are shown in **Supplementary Table 6**. Genetically predicted hypothyroidism using 5 SNPs was associated with a decreased $eGFR_{crea}$ [beta(SE); -0.0093(0.0026) ln(mL/min/1.73 m2),p=0.00036] in IVW analysis; this effect estimate was not significantly different from the equivalent estimate in the WGHS (p=0.71). The effect remained similar in WM analysis [-0.0076(0.0028), p=0.0069], and in MR Egger regression [-0.0070 (0.0123), p=0.61] with no heterogeneity (p=0.13) (**Table 3** and **Figure 2A**). A scatter plot depicting the relationship of the hypothyroidism SNP effects on hypothyroidism against the SNP effects on the $eGFR_{crea}$ demonstrated that there were no outlying SNPs influencing the regression (**Figure 2B**). The "leave-one-out" sensitivity analysis showed that the estimates were robust (**Supplementary Figure 2**).

Genetically predicted increased TSH (per SD(mIU/L)) using 17 TSH SNPs was not significantly associated with a decreased $eGFR_{crea}$ [beta(SE); -0.0047(0.0069) ln(mL/min/1.73

m2),p=0.49], in the IVW analysis, [-0.0092(0.0045)] in the WM analysis, and [-0.0169(0.0194)] in the MR Egger with heterogeneity p=$3.31*10^{-14}$ (**Table 3, Supplementary Figure 3**). We explored heterogeneity by scatter plot **(Supplementary Figure 3**), forest plot **(Supplementary Figure 4**), and "leave-one-out" analysis **(Supplementary Figure 5**). Removing the outlier SNP rs2396084 in VEGFA changed the direction of the estimate, but heterogeneity persisted (MR Egger, p=0.00050).

Neither genetically predicted hypothyroidism nor increased TSH was associated with $eGFR_{cys}$, CKD or UACR. Genetically predicted increased fT4 or increased TPOAb were not associated with $eGFR_{crea}$, $eGFR_{cys}$, CKD or UACR (**Table 3**).

Discussion

We demonstrated that genetically predicted hypothyroidism is associated with a decreased $eGFR_{crea}$ with similar and not statistically different point estimates in WGHS and CKDGen. In the CKDGen, the inverse variance weighted and median weighted analyses were statistically significant, but not in MR Egger. In the sensitivity analyses for the genetically determined hypothyroidism on $eGFR_{crea}$ in CKDGen, the MR Egger regression suggested pleiotropic effects of the genetic SNPs on $eGFR_{crea}$, but the intercept was close to zero and not statistically significant. Also, there were no individual data points having large influence on the regression as seen from scatter plots and "leave-one-out" analyses. The magnitude of the beta coefficients was similar for MR Egger regression and weighted median and in the same direction as the IVW estimate which was more negative; the latter likely suggests an inflated estimate. There was no evidence of heterogeneity in the MR Egger regression, the IVW, or the weighted median analyses. Since the single SNP analyses were all in the same direction and of similar magnitude, the MR Egger regression estimate is expected to be less precise(57, 58). Thus, the genetic estimates are in accordance with previous findings showing that

overt and subclinical hypothyroidism(3-8) and increased levels of TSH (3, 15-21) are associated with increased creatinine and decreased eGFR. Observationally and genetically predicted increased TSH within the reference range in WGHS and in CKDGen was not associated with decreased $eGFR_{crea}$ in contrast to previous results showing that TSH within the reference range is associated with reduced $eGFR_{crea}$ (3, 15-21).

The MR results for genetically predicted hypothyroidism or for increased TSH within the reference range and for development of CKD in WGHS and CKDGen were consistent with the observational results from WGHS, but were not so with the earlier observational estimates, which show that hypothyroidism(3, 4) or increased TSH within the reference interval (3, 15-19, 45) are associated with CKD. The reason for a non-significant result for CKD when the $eGFR_{crea}$ results were significant for genetically predicted hypothyroidism could be lower power with a binary outcome compared to a continuous outcome.

The most common endogenous cause of hypothyroidism is Hashimoto's thyroiditis, which is an autoimmune thyroiditis (AIT). The GWAS significant SNPs for hypothyroidism are involved in thyroid function (*FOXE1*) but more predominantly involved in immune function (*PTPN22, SH2B3, VAV3,* and *HLA* class 1) with a shared etiology with other autoimmune diseases(32). In the individual SNP analysis, the immune loci were more strongly associated with $eGFR_{crea}$ than *FOXE1*. Based on the MR results for hypothyroidism but not for TSH within the reference range, we hypothesize that autoimmune loci exert their downstream effects through a genetic predisposition/susceptibility to autoimmune destruction of the thyroid gland, leading to hypothyroidism with low thyroid hormone and a negative feedback on the hypothalamic-pituitary-thyroid axis with a subsequent rise in TSH being marker but not on the causal pathway. In turn, this may lead to circulating immunocomplexes of thyroglobulin and autoantibodies depositing in the glomeruli, and finally leading to decreased $eGFR_{crea}$ (37). A retrospective study of patients with

AIT showed that 85% had glomerular pathology: membranous glomerulonephritis (20%), focal segmental glomerulosclerosis (20%), IgA nephropathy (15%), chronic glomerulonephritis (15%), minimal change disease (10%), and amyloidosis (5%)(34). Distinct mechanisms have been hypothesized for the link between hypothyroidism and glomerular pathology, including deposition of thyroglobulin-antibodies and TPOAb(37); however, we did not find that genetically predicted increased TPOAb was associated with kidney function in CKDGen.

Whether increased thyroxine (T4) is associated with a decline or increase in kidney function (5, 6, 15, 17, 21, 44, 45) has been debated. However, our findings that neither observationally nor genetically predicted increased fT4 within the reference range in WGHS or CKDGen were associated with kidney function are in accordance with the proposed mechanism that it is an autoimmune destruction of the thyroid gland rather than thyroid function *per se* which is associated with reduced kidney function.

In the WGHS, the observational estimates suggested a reverse association between kidney function and thyroid function like in previous observational studies (40-43), but we observed no consistent reverse association of genetically predicted $eGFR_{crea}$ or CKD with thyroid function. These findings are in accordance with findings from two previous randomized double-blind placebo controlled trials which failed to show any improvement in kidney function when administering thyroid hormone to patients with ATN(59) or acute renal failure(60). Thus, the presence of the kidney-thyroid association in observational cross-sectional setting in WGHS could be due to reverse causation, but could also be explained by the occurrence of non-thyroidal illness. The laboratory findings in non-thyroidal illness are usually a low fT3 due to reduced D1 deiodinase activity with inappropriately low TSH possibly mediated by cytokine effects. The findings resemble diminished organ function (low fT3) and diminished hypothalamic function (low TSH). Thus, the T3 treatment

intervention in the RCT trial in ATN also had the negative outcome of a prolonged TSH suppression.

The *FOXE1* SNPs rs925489 (hypothyroidism) was associated with younger age at baseline. This could reflect selection bias, such that individuals who are more likely to be heterozygous or homozygous for these SNPs would have a lower tendency to participate. *FOXE1* (forkhead box E1), also known as TTF-2 (thyroid transcription factor 2)(52), is associated with thyroid cancer(61) and homozygous loss-of-function mutations is associated with congenital hypothyroidism due to thyroid dysgenesis and other developmental abnormalities(62).

Neither genetically predicted hypothyroidism or increased TSH within the reference range were associated with CKD, $eGFR_{cys}$, or UACR. The reason for the discrepancy between the findings for $eGFR_{crea}$ and $eGFR_{cys}$ may be lack of standardization of the cystatin C assays in the CKDGen consortium. Despite that a reference material for Cystatin C was introduced in 2010, increase in bias with concentration remains a major component of uncertainty likely due to problems associated with the implementation of traceability and problems related to calibration among some manufacturers(63), resulting in considerable heterogeneity in the $eGFR_{cys}$ statistical analyses. Furthermore, the filtration markers differ by their non-GFR determinants (age, gender, muscle mass, race) and by how they are handled by the kidney (filtration, re-absorption, secretion, catabolism)(64). Last but not least, a combined eGFR estimation using both creatinine and cystatin C is superior to eGFR estimations using either creatinine or cystatin C alone(64).

In conclusion, the genetically predicted hypothyroidism in this study is likely associated with decreased kidney function through an autoimmune mechanism; however, neither genetically predicted $eGFR_{crea}$ nor CKD is not associated with thyroid function. The bidirectional MR approach aids in identifying causal pathways leading to disease and eventually in identifying potential targets for therapeutic intervention in future randomized clinical trials.

Figures and figure legends

Figure 1. Overview of bidirectional Mendelian Randomization study of thyroid function and kidney function.

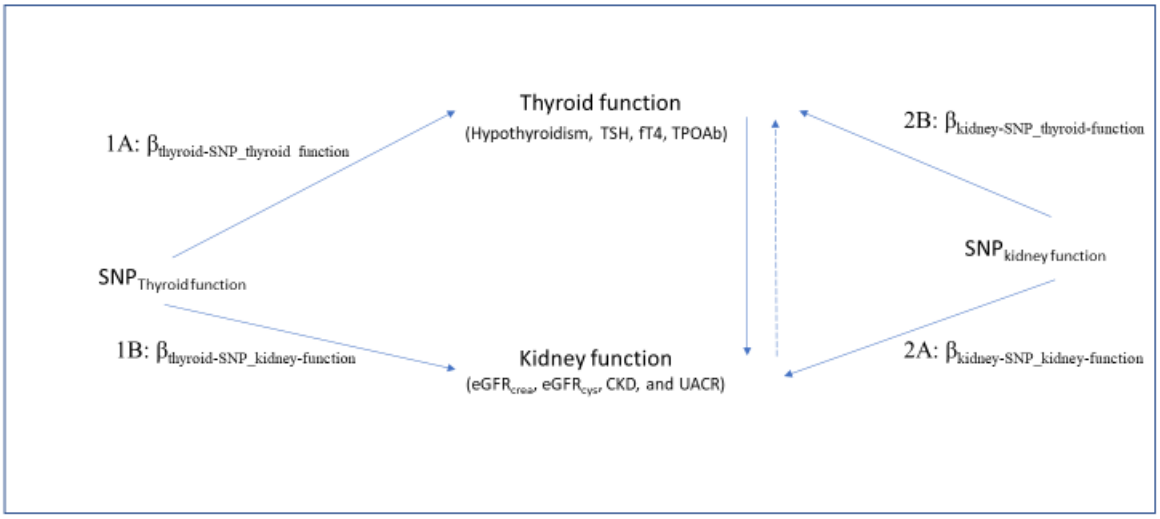


Figure 2

A. Individual instrumental variable estimates for each of the 5 hypothyroidism-associated SNPs with $eGFR_{crea}$ in CKDGen.

B. Comparison of the effect estimates of hypothyroidism related SNPs with their effect on $eGFR_{crea}$ in CKDGen.

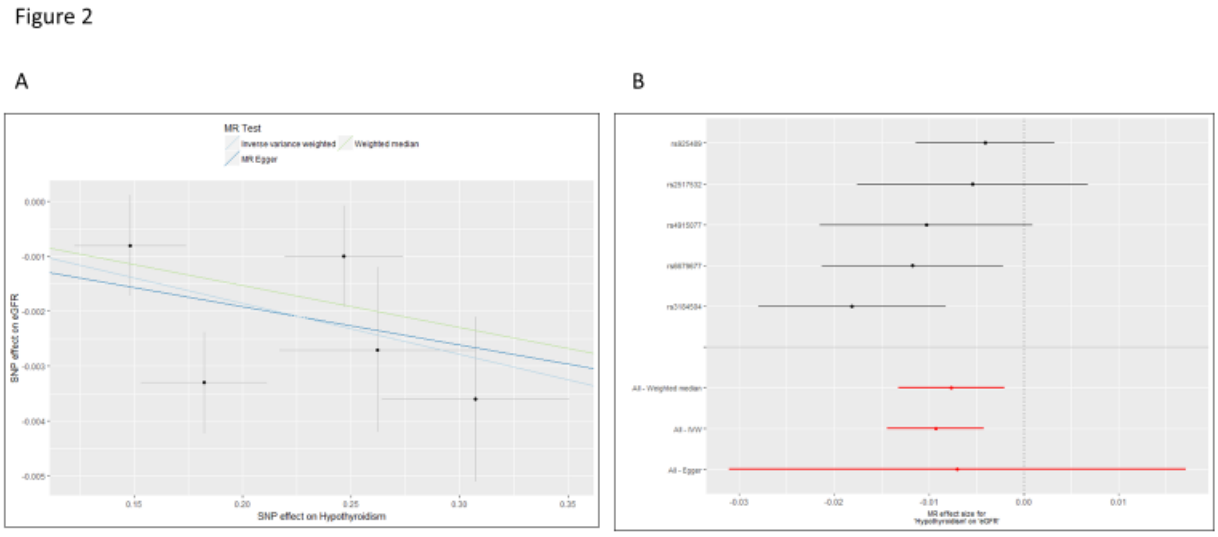


Table 1. Observational bidirectional associations between thyroid function and kidney function in Women's Genome Health Study (WGHS)

Exposure	Outcome	Regression model	Participants with TFT*	N	beta	SE	p-value
TSH	eGFRcrea*	Linear	All	3321	-0.003	0.001	2.32E-05
ln(TSH)	eGFRcrea*	Linear	All	3321	-0.015	0.003	5.55E-07
fT4	eGFRcrea*	Linear	All	3320	0.006	0.015	6.97E-01
ln(fT4)	eGFRcrea*	Linear	All	3320	0.000	0.018	9.87E-01
fT3	eGFRcrea*	Linear	All	3321	0.032	0.007	1.76E-06
ln(fT3)	eGFRcrea*	Linear	All	3321	0.121	0.023	1.26E-07
fT3/fT4 ratio	eGFRcrea*	Linear	All	3297	0.013	0.004	5.62E-04
ln(fT3/fT4 ratio)	eGFRcrea*	Linear	All	3297	0.071	0.017	4.56E-05
Hypothyroidism	eGFRcrea*	Linear	Hypothyroid and euthyroid	3042	-0.026	0.009	5.61E-03
TSH	eGFRcrea*	Linear	Euthyroid	2175	-0.009	0.005	6.82E-02
ln(TSH)	eGFRcrea*	Linear	Euthyroid	2175	-0.021	0.009	2.58E-02
fT4	eGFRcrea*	Linear	Euthyroid	2175	-0.047	0.033	1.60E-01
ln(fT4)	eGFRcrea*	Linear	Euthyroid	2175	-0.052	0.040	1.92E-01
fT3	eGFRcrea*	Linear	Euthyroid	2175	0.043	0.012	2.34E-04
ln(fT3)	eGFRcrea*	Linear	Euthyroid	2175	0.110	0.035	1.90E-03
fT3/fT4 ratio	eGFRcrea*	Linear	Euthyroid	2175	0.047	0.011	4.13E-05
ln(fT3/fT4 ratio)	eGFRcrea*	Linear	Euthyroid	2175	0.105	0.029	3.58E-04
TSH	CKD	Logistic	All	3321	0.022	0.008	9.87E-03
ln(TSH)	CKD	Logistic	All	3321	0.081	0.052	1.18E-01
fT4	CKD	Logistic	All	3320	0.087	0.248	7.27E-01
ln(fT4)	CKD	Logistic	All	3320	0.065	0.281	8.18E-01
fT3	CKD	Logistic	All	3321	-0.294	0.130	2.31E-02
ln(fT3)	CKD	Logistic	All	3321	-0.985	0.375	8.54E-03
fT3/fT4 ratio	CKD	Logistic	All	3297	-0.261	0.114	2.15E-02
ln(fT3/fT4 ratio)	CKD	Logistic	All	3297	-0.735	0.302	1.48E-02
Hypothyroidism	CKD	Logistic	Hypothyroid and euthyroid	3042	0.169	0.145	2.44E-01
TSH	CKD	Logistic	Euthyroid	2175	-0.015	0.084	8.62E-01
ln(TSH)	CKD	Logistic	Euthyroid	2175	0.067	0.162	6.78E-01
fT4	CKD	Logistic	Euthyroid	2175	0.736	0.555	1.85E-01
ln(fT4)	CKD	Logistic	Euthyroid	2175	0.862	0.672	2.00E-01
fT3	CKD	Logistic	Euthyroid	2175	-0.163	0.208	4.33E-01
ln(fT3)	CKD	Logistic	Euthyroid	2175	-0.361	0.589	5.40E-01
fT3/fT4 ratio	CKD	Logistic	Euthyroid	2175	-0.335	0.200	9.39E-02
ln(fT3/fT4 ratio)	CKD	Logistic	Euthyroid	2175	-0.699	0.485	1.49E-01
eGFRcrea*	TSH	Linear	All	3321	-1.975	0.466	2.32E-05
eGFRcrea*	ln(TSH)	Linear	All	3321	-0.518	0.103	5.55E-07
eGFRcrea*	fT4	Linear	All	3320	0.008	0.021	6.97E-01
eGFRcrea*	ln(fT4)	Linear	All	3320	0.000	0.018	9.87E-01
eGFRcrea*	fT3	Linear	All	3321	0.220	0.046	1.76E-06
eGFRcrea*	ln(fT3)	Linear	All	3321	0.072	0.014	1.26E-07
eGFRcrea*	fT3/fT4 ratio	Linear	All	3297	0.296	0.086	5.62E-04
eGFRcrea*	ln(fT3/fT4 ratio)	Linear	All	3297	0.074	0.018	4.56E-05
eGFRcrea*	Hypothyroidism	Logistic	Hypothyroid and euthyroid	3042	-0.590	0.219	7.01E-03
CKD	TSH	Linear	All	3321	1.144	0.339	7.52E-04
CKD	ln(TSH)	Linear	All	3321	0.126	0.075	9.57E-02
CKD	fT4	Linear	All	3320	0.004	0.015	8.13E-01
CKD	ln(fT4)	Linear	All	3320	0.001	0.013	9.59E-01
CKD	fT3	Linear	All	3321	-0.085	0.033	1.15E-02
CKD	ln(fT3)	Linear	All	3321	-0.031	0.010	1.92E-03
CKD	fT3/fT4 ratio	Linear	All	3297	-0.103	0.063	9.88E-02
CKD	ln(fT3/fT4 ratio)	Linear	All	3297	-0.034	0.013	1.08E-02
CKD	Hypothyroidism	Logistic	Hypothyroid and euthyroid	3042	0.172	0.143	2.29E-01

*TFT: thyroid function test

Units	
Hypothyroidism	yes(1) vs.no(0)
TSH	mIU/L
fT4	ng/dL
fT3/fT4	(ng/dL)/(ng/dL)
eGFR_creatinine	ln(mL/min/1.73 m2)
CKD	yes(1) vs.no(0)

Table 2. Bidirectional Mendelian Randomization associations between thyroid function and kidney function in Women's Genome Health Study (WGHS)

Instrument (G)	Exposure (X)	Outcome (Y)	MR method	N SNP	beta	SE	p-value	Pleiotropy, pvalue	Heterogeneity, pvalue
Hypothyroidism	Hypothyroidism	eGFR_creatinine	MR Egger	4	-0.0055	0.0145	7.40E-01	6.62E-01	6.69E-02
Hypothyroidism	Hypothyroidism	eGFR_creatinine	Weighted median	4	-0.0098	0.0053	6.74E-02	NA	2.54E-01
Hypothyroidism	Hypothyroidism	eGFR_creatinine	Inverse variance weighted	4	-0.0117	0.0068	8.35E-02	NA	2.54E-01
Hypothyroidism	Hypothyroidism	CKD	MR Egger	4	0.1469	0.1388	4.01E-01	7.15E-01	8.61E-01
Hypothyroidism	Hypothyroidism	CKD	Weighted median	4	0.0970	0.0875	2.68E-01	NA	9.57E-01
Hypothyroidism	Hypothyroidism	CKD	Inverse variance weighted	4	0.0977	0.0746	1.90E-01	NA	9.57E-01
TSH	TSH	eGFR_creatinine	MR Egger	23	-0.0184	0.0120	1.40E-01	5.76E-01	5.89E-02
TSH	TSH	eGFR_creatinine	Weighted median	23	-0.0070	0.0088	4.25E-01	NA	9.63E-02
TSH	TSH	eGFR_creatinine	Inverse variance weighted	23	-0.0130	0.0073	7.43E-02	NA	9.63E-02
TSH	TSH	CKD	MR Egger	23	0.1570	0.1663	3.56E-01	5.60E-01	2.56E-01
TSH	TSH	CKD	Weighted median	23	0.1254	0.1432	3.81E-01	NA	3.44E-01
TSH	TSH	CKD	Inverse variance weighted	23	0.0796	0.1014	4.32E-01	NA	3.44E-01
fT4	fT4	eGFR_creatinine	MR Egger	5	0.0262	0.0428	5.84E-01	4.94E-01	3.05E-01
fT4	fT4	eGFR_creatinine	Weighted median	5	-0.0037	0.0148	8.04E-01	NA	5.14E-01
fT4	fT4	eGFR_creatinine	Inverse variance weighted	5	-0.0059	0.0108	5.87E-01	NA	5.14E-01
fT4	fT4	CKD	MR Egger	5	-0.6001	0.6158	4.02E-01	4.27E-01	4.15E-01
fT4	fT4	CKD	Weighted median	5	-0.1150	0.2289	6.15E-01	NA	5.98E-01
fT4	fT4	CKD	Inverse variance weighted	5	-0.0562	0.1624	7.29E-01	NA	5.98E-01
GFR	eGFR_creatinine	Hypothyroidism	MR Egger	40	-2.3447	3.7583	5.36E-01	2.63E-01	4.06E-01
GFR	eGFR_creatinine	Hypothyroidism	Weighted median	40	2.1754	1.9252	2.58E-01	NA	4.38E-01
GFR	eGFR_creatinine	Hypothyroidism	Inverse variance weighted	40	1.6542	1.3212	2.11E-01	NA	4.38E-01
GFR	eGFR_creatinine	TSH	MR Egger	40	3.7604	1.8399	4.80E-02	1.05E-01	3.57E-01
GFR	eGFR_creatinine	TSH	Weighted median	40	1.5997	0.8926	7.31E-02	NA	3.26E-01
GFR	eGFR_creatinine	TSH	Inverse variance weighted	40	0.8958	0.6571	1.73E-01	NA	3.26E-01
GFR	eGFR_creatinine	fT4	MR Egger	40	-1.0183	1.7895	5.73E-01	4.36E-01	4.49E-01
GFR	eGFR_creatinine	fT4	Weighted median	40	0.8626	0.9650	3.71E-01	NA	5.12E-01
GFR	eGFR_creatinine	fT4	Inverse variance weighted	40	0.3021	0.6220	6.27E-01	NA	5.12E-01
CKD	CKD	Hypothyroidism	MR Egger	4	-0.0542	1.1124	9.66E-01	7.71E-01	9.37E-01
CKD	CKD	Hypothyroidism	Weighted median	4	-0.2698	0.7539	7.20E-01	NA	9.84E-01
CKD	CKD	Hypothyroidism	Inverse variance weighted	4	-0.3855	0.4958	4.37E-01	NA	9.84E-01
CKD	CKD	TSH	MR Egger	4	0.2887	0.5357	6.44E-01	2.87E-01	9.16E-01
CKD	CKD	TSH	Weighted median	4	-0.2753	0.6856	6.88E-01	NA	6.84E-01
CKD	CKD	TSH	Inverse variance weighted	4	-0.4008	0.2384	9.27E-02	NA	6.84E-01
CKD	CKD	fT4	MR Egger	4	-0.3926	0.8684	6.96E-01	8.62E-01	7.19E-02
CKD	CKD	fT4	Weighted median	4	-0.3634	3.2575	9.11E-01	NA	3.11E-01
CKD	CKD	fT4	Inverse variance weighted	4	-0.2391	0.3185	4.53E-01	NA	3.11E-01

Units	
Hypothyroidism	yes(1) vs.no(0)
TSH	SD(mIU/L)
fT4	SD(ng/dL)
TPOAb	SD(mIU/L)
eGFR_creatinine	ln(mL/min/1.73 m2)
CKD	yes(1) vs.no(0)
UACR	ln(mg/g)
Cystatic C	ln(mL/min/1.73 m2)

Table 3. Unidirectional Mendelian Randomization associations between thyroid function and kidney function in the CKDGEN consortium

Instrument (G)	Exposure (X)	Outcome (Y)	MR method	N SNP	beta	SE	p-value	Pleiotropy, pvalue	Heterogeneity, pvalue
Hypothyroidism	Hypothyroidism	eGFR_creatinine	MR Egger	5	-0.0070	0.0123	0.6091	0.8596	0.1312
Hypothyroidism	Hypothyroidism	eGFR_creatinine	Weighted median	5	-0.0076	0.0028	6.86E-03	NA	0.3704
Hypothyroidism	Hypothyroidism	eGFR_creatinine	Inverse variance weighted	5	-0.0093	0.0026	3.63E-04	NA	0.3704
Hypothyroidism	Hypothyroidism	eGFR_cystatinC	MR Egger	5	0.0241	0.0648	0.7345	0.6037	1.86E-07
Hypothyroidism	Hypothyroidism	eGFR_cystatinC	Weighted median	5	0.0037	0.0066	0.5779	NA	1.01E-05
Hypothyroidism	Hypothyroidism	eGFR_cystatinC	Inverse variance weighted	5	-0.0122	0.0145	0.3992	NA	1.01E-05
Hypothyroidism	Hypothyroidism	CKD	MR Egger	5	-0.0743	0.1555	0.6656	0.5946	0.4017
Hypothyroidism	Hypothyroidism	CKD	Weighted median	5	-0.0071	0.0457	0.8760	NA	0.6508
Hypothyroidism	Hypothyroidism	CKD	Inverse variance weighted	5	0.0153	0.0376	0.6845	NA	0.6508
Hypothyroidism	Hypothyroidism	UACR	MR Egger	5	-0.0255	0.0615	0.7064	0.8312	0.4725
Hypothyroidism	Hypothyroidism	UACR	Weighted median	5	-0.0185	0.0179	0.3009	NA	0.7491
Hypothyroidism	Hypothyroidism	UACR	Inverse variance weighted	5	-0.0116	0.0148	0.4323	NA	0.7491
TSH	TSH	eGFR_creatinine	MR Egger	17	-0.0169	0.0194	0.40	0.5113	3.31E-14
TSH	TSH	eGFR_creatinine	Weighted median	17	-0.0092	0.0045	0.04	NA	3.65E-13
TSH	TSH	eGFR_creatinine	Inverse variance weighted	17	-0.0047	0.0069	0.49	NA	3.65E-13
TSH	TSH	eGFR_cystatinC	MR Egger	17	0.0053	0.0316	0.87	0.8382	2.45E-07
TSH	TSH	eGFR_cystatinC	Weighted median	17	0.0061	0.0085	0.47	NA	2.02E-06
TSH	TSH	eGFR_cystatinC	Inverse variance weighted	17	-0.0008	0.0111	0.94	NA	2.02E-06
TSH	TSH	CKD	MR Egger	17	-0.0759	0.2035	0.71	0.7945	0.0011
TSH	TSH	CKD	Weighted median	17	-0.0244	0.0719	0.73	NA	0.0035
TSH	TSH	CKD	Inverse variance weighted	17	-0.0256	0.0718	0.72	NA	0.0035
TSH	TSH	UACR	MR Egger	17	-0.0266	0.0516	0.61	0.9677	0.4707
TSH	TSH	UACR	Weighted median	17	-0.0187	0.0263	0.48	NA	0.6082
TSH	TSH	UACR	Inverse variance weighted	17	-0.0285	0.0186	0.13	NA	0.6082
fT4	fT4	eGFR_creatinine	MR Egger	4	-0.0151	0.0421	0.75	0.7535	0.0275
fT4	fT4	eGFR_creatinine	Weighted median	4	-0.0027	0.0059	0.65	NA	0.1645
fT4	fT4	eGFR_creatinine	Inverse variance weighted	4	-0.0003	0.0074	0.97	NA	0.1645
fT4	fT4	eGFR_cystatinC	MR Egger	4	-0.0604	0.0752	0.51	0.4512	0.0676
fT4	fT4	eGFR_cystatinC	Weighted median	4	0.0022	0.0122	0.86	NA	0.1618
fT4	fT4	eGFR_cystatinC	Inverse variance weighted	4	0.0079	0.0153	0.60	NA	0.1618
fT4	fT4	CKD	MR Egger	4	0.4273	0.3622	0.36	0.3702	0.6417
fT4	fT4	CKD	Weighted median	4	0.0216	0.0926	0.82	NA	0.6897
fT4	fT4	CKD	Inverse variance weighted	4	0.0214	0.0766	0.78	NA	0.6897
fT4	fT4	UACR	MR Egger	4	-0.2211	0.2006	0.39	0.4352	0.1722
fT4	fT4	UACR	Weighted median	4	-0.0511	0.0438	0.24	NA	0.3281
fT4	fT4	UACR	Inverse variance weighted	4	-0.0311	0.0408	0.45	NA	0.3281
TPOAb	TPOAb	eGFR_creatinine	MR Egger	5	-0.0782	0.4065	0.86	0.9898	1.56E-07
TPOAb	TPOAb	eGFR_creatinine	Weighted median	5	-0.0331	0.0404	0.41	NA	3.36E-05
TPOAb	TPOAb	eGFR_creatinine	Inverse variance weighted	5	-0.0727	0.0682	0.29	NA	3.36E-05
TPOAb	TPOAb	eGFR_cystatinC	MR Egger	5	0.6079	0.5895	0.38	0.2456	0.0011
TPOAb	TPOAb	eGFR_cystatinC	Weighted median	5	-0.1309	0.0712	0.07	NA	0.0004
TPOAb	TPOAb	eGFR_cystatinC	Inverse variance weighted	5	-0.2250	0.1271	0.08	NA	0.0004
TPOAb	TPOAb	CKD	MR Egger	5	-0.3153	3.1020	0.93	0.8584	0.0645
TPOAb	TPOAb	CKD	Weighted median	5	-0.0048	0.5308	0.99	NA	0.2395
TPOAb	TPOAb	CKD	Inverse variance weighted	5	0.2757	0.5256	0.60	NA	0.2395
TPOAb	TPOAb	UACR	MR Egger	5	-1.2095	1.1822	0.38	0.3947	0.1496
TPOAb	TPOAb	UACR	Weighted median	5	-0.0055	0.2303	0.98	NA	0.2580
TPOAb	TPOAb	UACR	Inverse variance weighted	5	-0.0591	0.2244	0.79	NA	0.2580

Units	
Hypothyroidism	yes(1) vs.no(0)
TSH	SD(mIU/L)
fT4	SD(ng/dL)
TPOAb	SD(mIU/L)
eGFR_creatinine	ln(mL/min/1.73 m2)
CKD	yes(1) vs.no(0)
UACR	ln(mg/g)
Cystatic C	ln(mL/min/1.73 m2)
fT3/fT4	SD(ng/dL)/SD(ng/dL)

Supplementary figures and tables

All supplementary files can be viewed in this shared google drive folder:

https://drive.google.com/open?id=136hwnwMlSmqhKIWGcjN1n7bT6SVcj9EN

Project 2: Thyroid function and atrial fibrillation – Mendelian Randomization

Draft 2018-MARCH-16

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Abstract

Background: Increased fT4 and decreased TSH are associated with increased risk of atrial fibrillation (AF) in observational studies, but causal involvement is unclear.

Objective: To test the causal effect of fT4, TSH, hypothyroidism, and TPOAb on AF using Mendelian Randomization (MR) in the AF Genetics (AFGen) Consortium.

Methods: Using study-level data, we created a genetic risk score fT4 (GRS_{fT4}) with genome-wide (GWAS) significant single-nucleotide polymorphisms (SNPs). The GRS_{fT4}-AF association was tested by meta-analysis among 11 studies including 56,912 participants (2093 prevalent and 5586 incident AF cases), accounting for TSH. We also performed a two-sample MR approach using GWAS instruments for fT4, TSH, TPOAb, hypothyroidism, and AF, the latter including 17,931 individuals with AF from the AFGen Consortium.

Results: In study-level analysis, each increment in GRS_{fT4} was associated with an increase in fT4 by 0.02 ng/dL (95%CI: 0.01-0.02) in multivariable analysis including TSH adjustment, but not with prevalent or incident AF (OR[95%CI]=1.03[0.93-1.14], HR[95%CI]=0.98 [0.94-1.03]). In summary-level analysis, there was weak instrument bias for fT4, hypothyroidism, and TPOAb, none of which was associated with AF. However, genetically predicted TSH from summary statistics was associated with AF with an inverse variance weighted OR (95% CI)=0.92(0.86-0.98, p=0.015) that was robust to test of pleiotropy.

Conclusions: MR analysis suggested that increased TSH may have a causal role in decreased risk of AF. There was no support for causality for fT4, TPOAb, or hypothyroidism in AF, but there was evidence of weak instrument bias and pleiotropy.

Introduction

The pituitary thyroid stimulating hormone (TSH) regulates several enzymes in the synthetic pathway of the production and secretion of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) which have a negative feedback on TSH(65, 66). The thyroid hormones are transported in the blood mostly bound to proteins, but the free fraction is considered a more accurate reflection of thyroid status because the total concentrations are influenced by changes in thyroid binding proteins as well as hypothalamic-pituitary-thyroid axis regulation(67). T4 is biologically inactive but is converted into an active hormone, T3, by deiodinases D1 and D2(68, 69), and then transported into the cardiomyocyte where it binds to nuclear receptors(70). In most observational studies, TSH and free or total T4 rather than T3 are measured.

Observational follow-up studies have shown that overt primary hyperthyroidism(22, 23), subclinical hyperthyroidism(23, 24), and increased free T4 (fT4) within the reference range(25) are associated with increased risk of atrial fibrillation (AF). Risk of AF is highest at the time of diagnosis of hyperthyroidism, but risk persists several decades after diagnosis and even after intervention with antithyroid treatment (drugs, radioiodine, or thyroidectomy)(22, 71-73). Likewise, overt and subclinical hypothyroidism are associated with reduced risk of AF(23). Being female and being a smoker both independently increase risk of developing hyperthyroidism (74, 75), and smoking is also associated with increased risk of AF(76).

The observation that AF risk persists despite antithyroid treatment, raises the question if fT4 is on the causal pathway or instead a biomarker for the hyperthyroid-AF association, for example related to a shared latent condition. Moreover, the feedback loop of fT4 onto TSH complicates causal inference of fT4 on AF. A Mendelian Randomization (MR) design, using genetic variants as proxies for hyperthyroidism or fT4, may be used to indirectly support or refute causality for the association of hyperthyroidism with AF(51). The assumption in MR is that a random assortment of

exogenous fT4 alleles at conception ensures a balanced distribution of confounders across the genotypes, thereby circumventing reverse causation and mimicking a randomized trial of fT4. If fT4 is directly involved in the development of AF, then inherited genetic variation influencing fT4 should affect AF risk in the direction and magnitude consistent with the observational associations. This inference assumes a lack of horizontal pleiotropic effects of the genetic variation on AF risk, i.e. that the instrument does not influence AF through other intermediate phenotypes other than the exposure investigated(1). Recently, genome-wide association studies (GWAS) or whole genome sequencing studies have identified common single nucleotide polymorphisms (SNPs) for variation in concentrations of TSH and free T4 (fT4) within the normal range, for hypothyroidism, and for thyroid peroxidase antibodies (TPOAb, a marker of autoimmune thyroid suppression)(29-32, 53) among adults of European ancestry. In total, 22% of the variance in fT4 is explained by the common SNPs for fT4(31). Also, a moderately rare variant (minor allele frequency of 0.4%) in the transthyretin gene *TTR* (rs28933981, Thre139Met) resulting in a tighter binding of thyroxine (T4) to the binding protein transthyretin (TTR)(77) has a large effect on fT4 levels(31). No GWAS significant loci have been identified for hyperthyroidism among individuals of European ancestry.

In this study, we used the MR design to address the causality between genetically predicted fT4, TSH, hypothyroidism, or TPOAb, and AF in the AF Genetics (AFGen) Consortium. We used study-level data from 11 participating studies to investigate the genetic risk score for fT4 and the rare variant rs28933981 allele score as instrumental variables for AF, while also exploring the fT4 effect conditional on potential independent effects of TSH. To gain additional power for fT4 analysis and assess causality of other thyroid measures, we also used summary-level data from GWAS in two-sample MR to investigate genetically predicted fT4, TSH, hypothyroidism, or TPOAb as instruments as predictors of AF, although these resources for these analyses could not account for conditional effects.

Methods

Participants

Study-level data

Studies participating in the AF Genetics (AFGen) Consortium were invited to participate in a MR study of fT4 and AF. Studies were eligible if they could identify at least one fT4 SNP. Eleven studies participated with 56,912 participants, including 7,679 AF cases (2093 prevalent and 5586 incident), and 49,233 referents. For MR studies using study-level data, we performed meta-analyses across the 11 studies of results generated according to a standard analysis plan. We included only analysis from European study populations as the previous GWAS of thyroid pertained to this group. The institutional review boards at each participating institution approved the individual studies.

We used fT4 (ng/dL=pmol/L(12.9)) and TSH (mIU/L) measured at baseline. fT4 was measured on all or a random subset of the individuals in each collaborating study (**Supplemental Table 1**). We used covariates relevant for AF including age (years), sex (0: women, 1: men), clinical center (if applicable), first three principal components of sub-European population structure (if available) or more dependent on study, height (cm), smoking status (1: current , 0: never/previous), BMI (kg/m^2), systolic blood pressure (in mmHg), diastolic blood pressure (in mmHg), antihypertensive medication use (yes/no), diabetes mellitus (yes/no), alcohol intake (2 or more drinks per day (1), vs. less than 2 drinks per day(0)), prevalent heart failure (cohort-specific definition) (yes/no), prevalent coronary heart disease (CHD) (cohort-specific definition) (yes/no), TSH (mIU/L) (**Table 1)**.

Two-sample MR approach using summary-level data

In the two-sample MR approach the instrument-exposure associations came from published summary statistics in published GWAS among individuals of European ancestry for TSH, fT4, hypothyroidism, or TPOAb(29-32, 53). Summary estimates for instrument-outcome associations came from a published AFGen Consortium GWAS of the common variants in 31 studies including 17,931 individuals with AF and 115,142 referents(27), and a rare variant association study (RVAS) including 22,346 cases and 132,086 referents(27), all of European ancestry.

Genotyping and genetic risk score

Existing study level genotype data sets were used for the analysis. In all cases, genotypes were derived from array-based genotyping platforms followed by imputation. Cohort specific genotyping methodology on genotyping is described elsewhere(27, 78, 79) and in **Supplemental Table 2.**

Study-level analysis

Five SNPs (4 common, i.e. MAF>1%, 1 rare) from two previous GWAS[18,19] were used as genetic instruments for fT4 in the reference range in MR. We used *DIO1*(rs2235544)(31), *AADAT*(rs7694879)(31), *LHX3*(rs11103377)(31) from Taylor(31) as these were the most recent and determined by direct genotyping on the exome chip, and therefore had high quality. We also used the *FOXE1*(rs7045138)(29) variant which only appeared in Porcu(29). The *DIO1* SNP was identical in Taylor and Porcu(29, 31) whereas the *AADAT* and *LHX3* SNPs were different in the two studies but in LD, r^2=0.69 and r^2=0.78, respectively. A fifth variant, *B4GALT6* rs113107469, was not included as this is in weak LD with the *TTR* rare variant, and when analyzing the *B4GALT6* variant on fT4 conditioning on the *TTR* variant, the association disappears(31). SNPs potentially involved in the feedback loop of fT4 and TSH were investigated by Porcu(29) who showed that

none of the fT4 SNPs was associated with TSH at a GWAS significant level, but the A-allele in *LHX3* was nominally associated with elevated TSH(29).

To study the effects of the 4 common SNP instruments on atrial fibrillation, each study created a genetic risk score for fT4 (GRS_{fT4}) by using the allele count or the maximum likelihood dose for imputed alleles summed at each of the 4 SNPs and weighted by the effect size (beta-coefficient):

GRS_{fT4} = [0.154*DIO1(rs2235544_A) + 0.137*AADAT (rs7694879_T) + 0.087*LHX3 (rs11103377_G) + 0.098*FOXE1(rs7045138_T)] * 4/0.476.

If the proxy rs1443434_T was used instead of rs7045138_T, the FOXE1 was replaced with 0.08*FOXE1(rs1443434_T) in the equation. Each study also provided separate analyses for each of the individual 4 common variants.

The analysis of the rare variant *TTR* Thr139Met (rs28933981)(31) on atrial fibrillation were done separately and was only included if genetic information came from exome-chip data.

Summary level data

In the summary level analysis, the genetic instruments for fT4 were the same as in the study level analysis. Common SNPs have been identified in GWAS among Europeans for TSH (N=24) concentrations in the reference range(29, 31), for TPOAb concentration (N=5)(30, 53), and for hypothyroidism (N=5)(32, 52). Of these, we identified 23 TSH, 5 fT4, 4 hypothyroidism SNPs, and 4 TPOAb SNPs in the summary statistics of the GWAS for AF from the AFGen Consortium(27). The AFGen GWAS comprised 17,931 individuals with atrial fibrillation and 115,142 referents across 31 studies(27). SNPs selected for each thyroid phenotype were not in LD(29, 31). We looked up the rare variant rs28933981_T in *TTR* in the summary statistics from the rare variant association study (RVAS) for AF from AFGen including 22,346 cases and 132,086 referents(27).

Outcome

The AF diagnosis was a cohort-specific definition including physician adjudication, questionnaire self-report, electrocardiography, and diagnosis codes for AF or flutter (International Classification of Diseases, Ninth Revision, Clinical Modification 427.3, 427.31 or 427.32; International Classification of Diseases, 10th Revision I48) present in hospitalization discharges or death certificates (**Supplemental Table1**). For study-level data, we investigated prevalent and first incident AF separately; if incident AF was used, baseline AF was excluded. In the AF GWAS used for summary level analysis, prevalent and incident AF combined through meta-analysis.

Statistics -Study level data

First, we examined the observational associations of fT4 or TSH with prevalent or incident AF using logistic or Cox regression, respectively. Second, we used the GRS_{fT4} and the individual fT4 SNPs separately as genetic instruments for all analyses of instrument-fT4 and instrument-AF associations. The association of genetic instruments with fT4 was assessed by using linear regression. The individual SNP instrument strength is measured using an approximated F-statistic reflecting the magnitude and the precision of the genetic effect: $F=GX^2/GX(SE)^2$, where GX is the per-allele genetic effect on exposure and GX(SE) is the standard error of GX(80, 81). The recommended threshold for the F-statistic is 10 or above(51).

We used logistic regression for prevalent AF and Cox regression model for incident AF. In the prospective cohorts, follow-up time was defined from study baseline until the first occurrence of AF, death, loss to follow-up, or end of study period. Four models were considered. Model 1(simple) included adjustment for age, sex, principal components (PCA, 3 first), and, if appropriate, clinical center. Model 2 (multiadjustment) was model 1 additionally adjusted for factors which could

confound the fT4-AF relationship including smoking status, alcohol intake, BMI, height, systolic blood pressure, diastolic blood pressure, antihypertensive medication, prevalent diabetes mellitus at baseline, prevalent heart failure at baseline, and prevalent CHD at baseline. Model 3(multi+TSH) was model 2 additionally adjusted for TSH (continuous, mIU/L) as a pleiotropy test. Model 4(multi+fT4) was model 2 additionally adjusted for fT4 (continuous, ng/dL) as a mediation test. We ran all analyses for men and women combined and separately, and for smokers and non-smokers combined and separately. Each collaborating study performed analyses separately and used their preferred statistical program (SAS, R, or STATA) for cohort-specific analyses.

The primary authors collected and calculated meta-analyses across studies. Meta-analyses were performed with STATA SE 14.0 (Stata Corp., College Station, TX, USA). Random (DerSimonian and Laird) effects pooled odds/hazard ratios or beta-coefficients were calculated for each analysis. Heterogeneity was assessed by Cochran's Q statistic test and I^2-statistical analysis. Egger's test of publication bias was used as a test of participation bias.

Statistics – summary-level MR

Published GWAS summary statistics were used for fT4, TSH, hypothyroidism, and TPOAb(29, 31) (**Supplementary Table 3**) and GWAS summary statistics from AFGen were used to determine the instrument-exposure and the instrument-AF relationships, respectively(27) (**Supplementary Table 4**). MR was performed with the R packages MRInstruments and TwoSampleMR and the Stata package *mrrobust*. We present the MR results in SD units of the biomarkers. For the individual MR estimates for each of the SNPs we applied the ratio estimator with the standard error calculated using the delta method(82). The IVW MR estimate was fitted as a weighted linear regression constraining the intercept at the origin and with the variance of the estimate being the inverse of the sum of the weights as in a fixed effect meta-analysis. The IVW method assumes that all genetic variants are valid instruments with no pleiotropy(57). Pleiotropy is the situation when instruments

influence AF through multiple intermediate phenotypes other than the exposure investigated. Presence of pleiotropy can be assessed by meta-analysis of the individual instrument MR Wald estimates; the between-instrument heterogeneity Q-statistic and the I^2_{MR} index will then describe the percentage of total variation in MR estimates across instruments that arises because of heterogeneity rather than chance(83). The individual SNP instrument strength is measured using an approximated F-statistic reflecting the magnitude and the precision of the genetic effect: $F=GX^2/GX(SE)^2$, where GX is the per-allele genetic effect on exposure and GX(SE) is the standard error of GX(80, 81). The recommended threshold for the F-statistic is 10 or above(1, 51).

Sensitivity analyses:

We performed sensitivity analyses with MR Egger regression, a weighted median MR estimator(57), and "leave one out" forest plots. MR Egger and weighted median MR account for potential horizontal pleiotropic bias (i.e. effects on AF not mediated by a candidate mediator) from invalid instruments by assessing the possibility that the ratio of effects on the mediator and outcome (i.e. AF) across genetic instruments is not constant(57). MR Egger tests whether effects of genetic variants are independent of instrumental strength(57). Potential bias is assessed by MR Egger regression as the magnitude and the significance of the intercept term, reflecting a deviation from a uniform ratio of instrument associations with thyroid measure and AF across the instruments(84). Potential violation of the "NO Measurement Error" (NOME) assumption in the MR-Egger analysis is quantified by I^2_{GX} which describes the degree of heterogeneity in the instrument-exposure at AF estimates generated by the different instruments(83). An I^2_{GX} statistic of 90% indicates that the likely bias due to measurement error in the MR-Egger slope is approximately 10%. If I^2_{GX} statistic is less than 90% this indicates weak instrument bias by violation of the "NO Measurement Error" (NOME) assumption.

In the weighted median approach, MR estimates for each instrument separately are ordered by the estimate value and the fraction of the distribution of each of these estimates is proportional to the inverse of their variances. The method assumes that 'instruments' representing more than 50% of the weight comes from valid instruments(57). The median of this distribution is the MR weighted median estimator, and confidence intervals are generated using a parametric bootstrap method (1000 replications)(57). "Leave-one-out analysis" was performed by repeating the MR-analysis excluding one instrument at a time to estimate if the MR result is driven by a single SNP. Finally, to visualize potential bias we examined funnel plots comparing the instrument strength (1/SE) for each SNP against the causal MR estimates and looked for asymmetry as in indication of potential directional pleiotropy from causal estimates of weaker variants(84).

Results

Study-level results

Characteristics of studies:

Eleven studies participated with 56,912 individuals, of which 2,093 had prevalent and 5,586 had incident AF, respectively (**Table 1**). Six studies were case-control and 8 studies prospective. Mean age ranged from 53-76 years, mean BMI ranged from 26-29, smoking prevalence ranged from 9-35%. Baseline fT4 levels were available in 3 of 6 case-control studies and 4 of 8 prospective studies. baseline TSH levels were available in 4 of 6 case-control studies and 5 of 8 prospective studies.

Observational association of fT4 and TSH with AF:

In meta-analysis of study-level results, the odds ratio for prevalent AF and hazard ratio for incident AF were 2.80 (95%CI:1.41-5.54) and 1.71 (1.09-2.70) in multivariable adjusted analyses, respectively, and were 2.86 (95%CI:1.31-6.22) and 1.90 (1.09-3.31) in multivariable+TSH adjusted analyses, respectively (**Figure 1**); results were similar in smoking-stratified and gender-stratified analyses (**Supplementary Figure S1**). TSH was not associated with AF in multivariable analyses(**Figure 1**), except for a hazard ratio of 1.01 (1.00-1.02) with incident AF in the multivariable+fT4 adjusted analysis (**Figure 1**) which was largely driven by females and nonsmokers (**Supplementary Figure S2**). I^2 heterogeneity ranged from 64-79% in fT4 analyses, and from 0%-32% in TSH analyses.

Genetic instruments and fT4 and TSH:

In meta-analysis, study level data showed that fT4 concentrations increased by 0.02 ng/dL (95%CI: 0.01-0.02) for each unit increment in the GRS_{fT4} in the simple model (**Figure 2)** with no evidence of

participation bias (p=0.88) and with an instrument strength of F=95**;** results were similar in multiadjusted and multiadjusted+TSH models (**supplementary Figure 3**). In meta-analysis of the 6 studies that had genotype information for the rare variant, rs28933981 in the TTR gene, fT4 concentrations increased by 0.19 ng/dL (0.13-0.25) for each additional T allele (**Figure 2**); results were similar in all models (**Supplementary Figure S3**).

TSH concentrations increased by 0.10 mIU/L (0.03-0.17) for each unit increment in the GRS_{fT4} in the simple model (**Figure 2**) but with evidence of participation bias (p=0.003) and instrument strength of F=7; results were similar in multiadjusted and multiadjusted+fT4 models (**Supplementary Figure 3**). The rare variant rs28933981 was borderline associated with decreased TSH in the simple and multiadjusted model (**Figure 2, Supplementary Figure 3**) and with increased TSH in the multiadjusted+fT4 model(**Supplementary Figure S3**).

Genetic instruments and AF:

Meta-analysis of study-level results showed that neither the GRS_{fT4} nor the rare variant rs28933981 was associated with prevalent or incident AF in any of the models (**Figure 3, Supplementary Figure S4-S7**).

Summary-level results from AFGen

Summary statistics for genetic association with fT4 and TSH were from GWAS by Taylor et al.(31) and Porcu et al.(29), respectively. The GWAS for AF included 31 studies with 17,931 individuals with AF and 115,142 controls(27) . The F-statistics for the published instruments from the GWAS for the intermediate risk factors ranged from 32-141 for TSH, 34-132 for fT4, 34-83 for hypothyroidism, and 10-19 for TPOAb, which are all above the recommended threshold of 10(51) (**Supplementary Table 3**).

In two-sample IVW MR analysis, a genetically predicted 1-SD increase in TSH (mIU/L) was associated with AF with an IVW MR estimate of the OR(95%CI) of 0.92(0.86-0.98, p=0.015), a weighted median MR OR (95%CI) of 0.89(0.81-0.97, p=0.009), and an MR-Egger OR (95%CI) of 0.89 (0.73-1.08, p=0.231) (**Table 2, Figure 4**). There was evidence of moderate directional horizontal pleiotropy with an I^2_{MR} (95%CI) of 19 (0-49)% (**Table 2**). The $I^2_{GX,}$statistic was 88% (**Table 2**) reflecting a relative bias of 12% towards the null and suggesting that there is no major evidence of measurement error biasing the MR-Egger analysis (**Table 2**). The leave-one-out analyses showed that there was no single SNP driving the association (**Supplementary Figure S8A**), or funnel plot asymmetry (**Supplementary Figure S8B**).

None of genetically predicted fT4, hypothyroidism, or TPOAb was associated with AF in MR IVW analysis. The NOME was violated for these associations with I^2_{GX} values of 75% for fT4, 55% for hypothyroidism, and 0% for TPOAb indicating weak instrument biasand there was evidence of pleiotropy with I^2_{MR} values of 81% and 53% for fT4 and hypothyroidism, respectively (**Table 2**, **Supplementary Figure S9-S14**). The IVW mendelian odds ratio estimate for AF for the rare fT4 variant rs28933981_T was 1.05 (0.81-1.36).

Discussion

We used the MR design to investigate causality between genetically predicted fT4, TSH, hypothyroidism, or TPOAb and AF in the AF Genetics (AFGen) Consortium. Using summary-level data from GWAS in two-sample MR analysis we showed that genetically predicted increased TSH is causally associated with decreased risk of AF and robust to test of directional horizontal pleiotropy, even when the observational finding was null. We were not able to demonstrate that genetically predicted increased fT4 is causally associated with increased risk of AF using study-level data from 11 studies or summary-level data, even the observational finding showed an increased risk. Furthermore, we were not able to demonstrate causality between the rare variant rs28933981 in TTR and AF in the study-level data, or between instruments for hypothyroidism or TPOAb and AF in summary-level data.

Use of weak instruments violating the "no measurement error" (NOME) assumption can bias MR estimates towards the observational estimate in study-level analyses and towards the null in summary-level analyses(1). In the study-level analyses, we used a weighted genetic risk score for fT4 which passed the F-statistic threshold ≥10 (F=95)(51). In the summary-level IVW MR, weak instrument bias was not detected using the F-statistics which was ≥10 for all instruments. In the summary-level MR Egger method using the I^2GX for regression dilution bias, weak instrument bias violating the NOME assumption was detected for fT4, hypothyroidism, and TPOAb which may explain the null-findings for these instruments. Weak instrument bias may occur if SNPs are not genome-wide significant or selected from less-powered GWAS(1). However, we used only GWAS significant SNPs, but the previous GWAS may not have captured all possible thyroid function SNPs.

We employed different available MR methods with different assumptions and interpretations. The findings across different MR methods were consistent in magnitude and

direction for genetically predicted TSH on AF using summary-level data; the MR-Egger which has less statistical power compared to IVW and weighted median methods, was not significant, but the analysis suggested no pleiotropy(84).

Using study-level data, we demonstrated that some of the genetic instruments for fT4 (genetic risk score, LHX3, AADAT, and FOXE1) had pleiotropic effects as they associated with increased TSH. Using summary-level data, MR Egger demonstrated pleiotropy for the genetic instruments for fT4 and hypothyroidism, which may explain the null-findings for these instruments.

The GWAS's of fT4 and TSH, from which we identified the SNPs in this study, were conducted in individuals with normal thyroid function(29, 31). However, in these GWASs the GWAS TSH SNPs also contributed to variation outside the reference range, indicating involvement in thyroid dysfunction, but this was not the case for the GWAS fT4 SNPs(29, 31). Therefore, our findings that genetically increased TSH was associated with decreased risk of AF may apply for TSH increase within the reference interval as well as for TSH increase (i.e. hypothyroidism) outside the reference interval. But for genetically predicted fT4, our findings are not applicable outside the fT4 reference range, which could also explain the lack of association with AF.

Our finding that a genetically predicted 1 SD increment in TSH was associated with 8-11% reduced odds of AF corresponds to the observational incidence rate ratio of 0.87 (0.79 to 0.97) for subclinical hypothyroidism in a recent large Danish cohort study (~600,000 individuals)(23). However, in our observational study and in another previous individual patient-data meta-analysis of 11 studies (N=30,000) increased TSH was not associated with decreased risk of atrial fibrillation(25), but these studies could be subject to confounding.

Observational studies are lacking for the association of changes in T3 with AF, despite the fact that the cardiac myocytes lack deiodinase activity and that T_3, but not T_4, is transported into the myocyte(70). Thus, our finding that genetically determined fT4 is not associated with AF is in

accordance with cellular mechanisms. It is thus likely that fT4 associations with AF in observational studies are confounded and driven instead by T3.

Like randomized double-blind clinical trials, MR studies are randomized biological double-blind natural trials which may mimic effects of pharmacological intervention and are not prone to reverse causation or confounding, However, a MR study is not equivalent to and cannot replace a RCT. Antithyroid drugs are thionamides (carbimazole, methimazole, and propylthiouracil), which act by inhibiting synthesis of thyroid hormones(74). Propylthiouracil also inhibits T4 conversion to T3 by inhibiting deiodinase-1. So far, the functions of the fT4 genes identified are located to the brain (*AADAT*, *B4GALT6*), pituitary (*LHX3*, *B4GALT6*), thyroid (*FOXE1*), thyroid transport (*TTR*), and peripheral deiodination of T4 to T3 (*DIO1*). Thus, only the *FOXE1* and the *DIO1* may resemble actions of thionamides, but none of the SNPs in the genes were significantly associated with AF.

In conclusion, MR analysis suggested that increased TSH may have a causal role in decreased risk of AF. Genetic instruments for fT4, TPOAb, or hypothyroidism demonstrated weak instrument bias and pleiotropy.

Figures

Figure 1. Meta-analyses of fT4 or TSH on atrial fibrillation – Study level

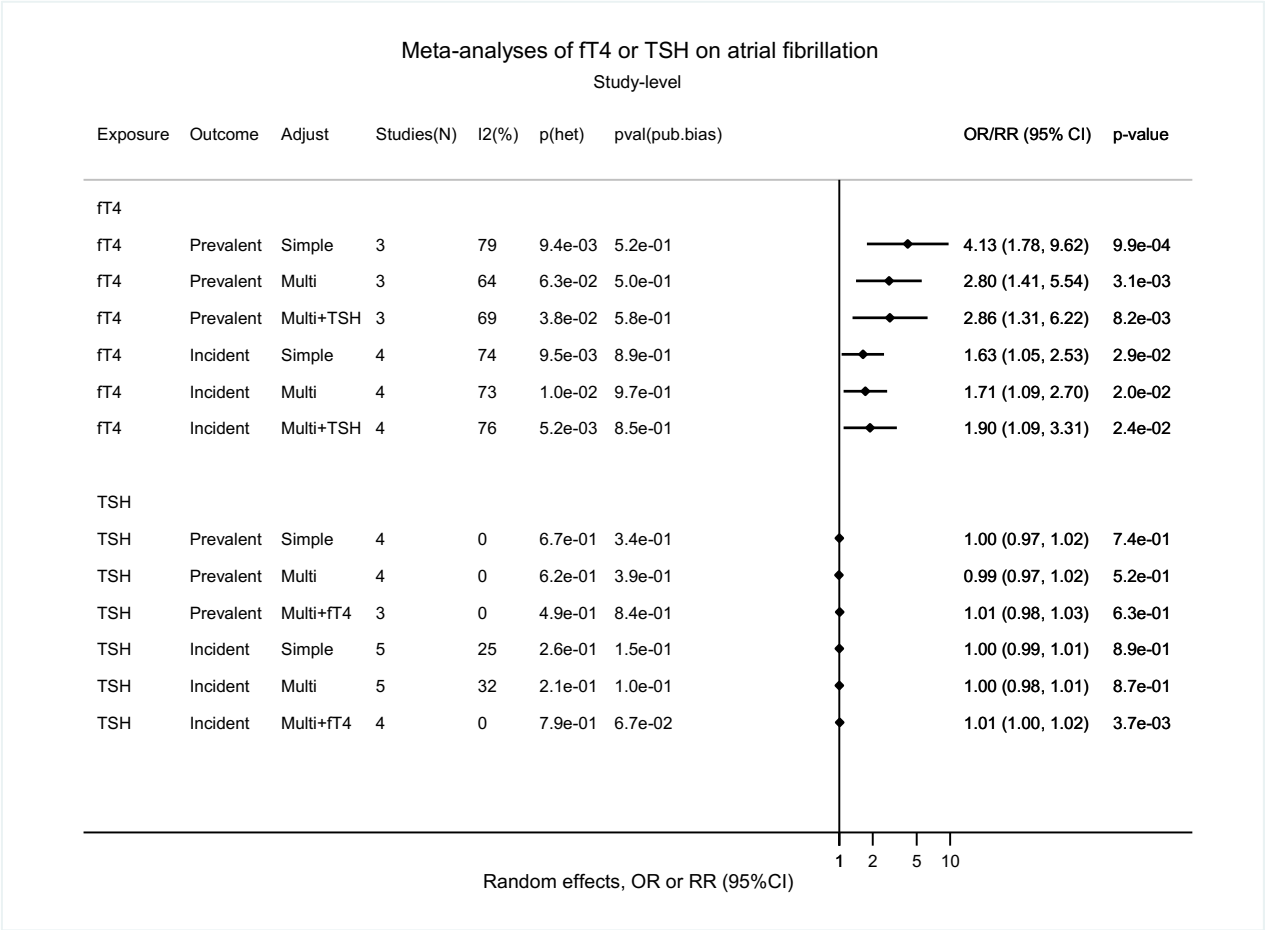


Figure 2. Meta-analyses of fT4 instruments on fT4 and TSH – Study-level. GRS(fT4): weighted genetic risk score for fT4.

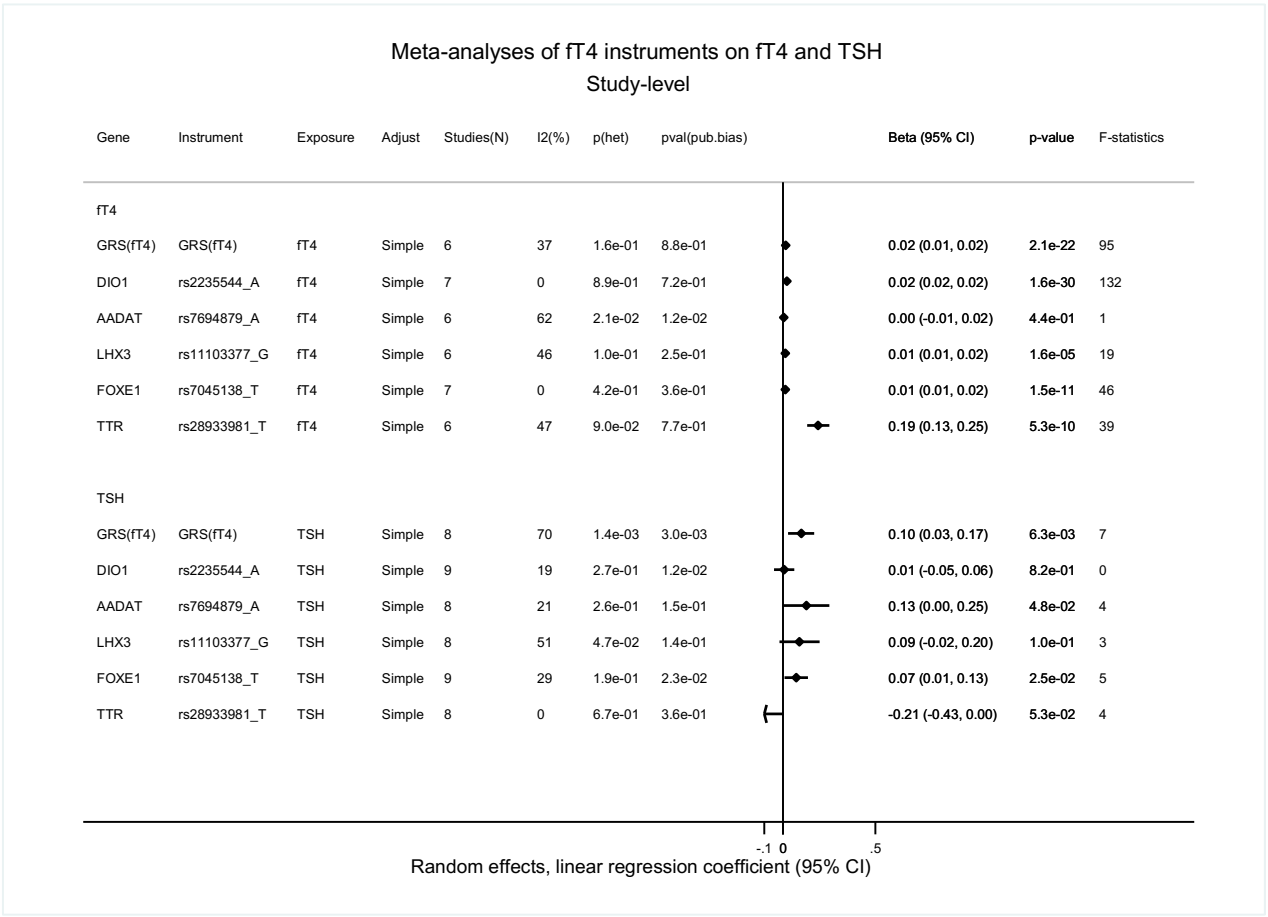


Figure 3. Meta-analyses of fT4 instruments on atrial fibrillation – Study level. GRS(fT4): weighted genetic risk score for fT4.

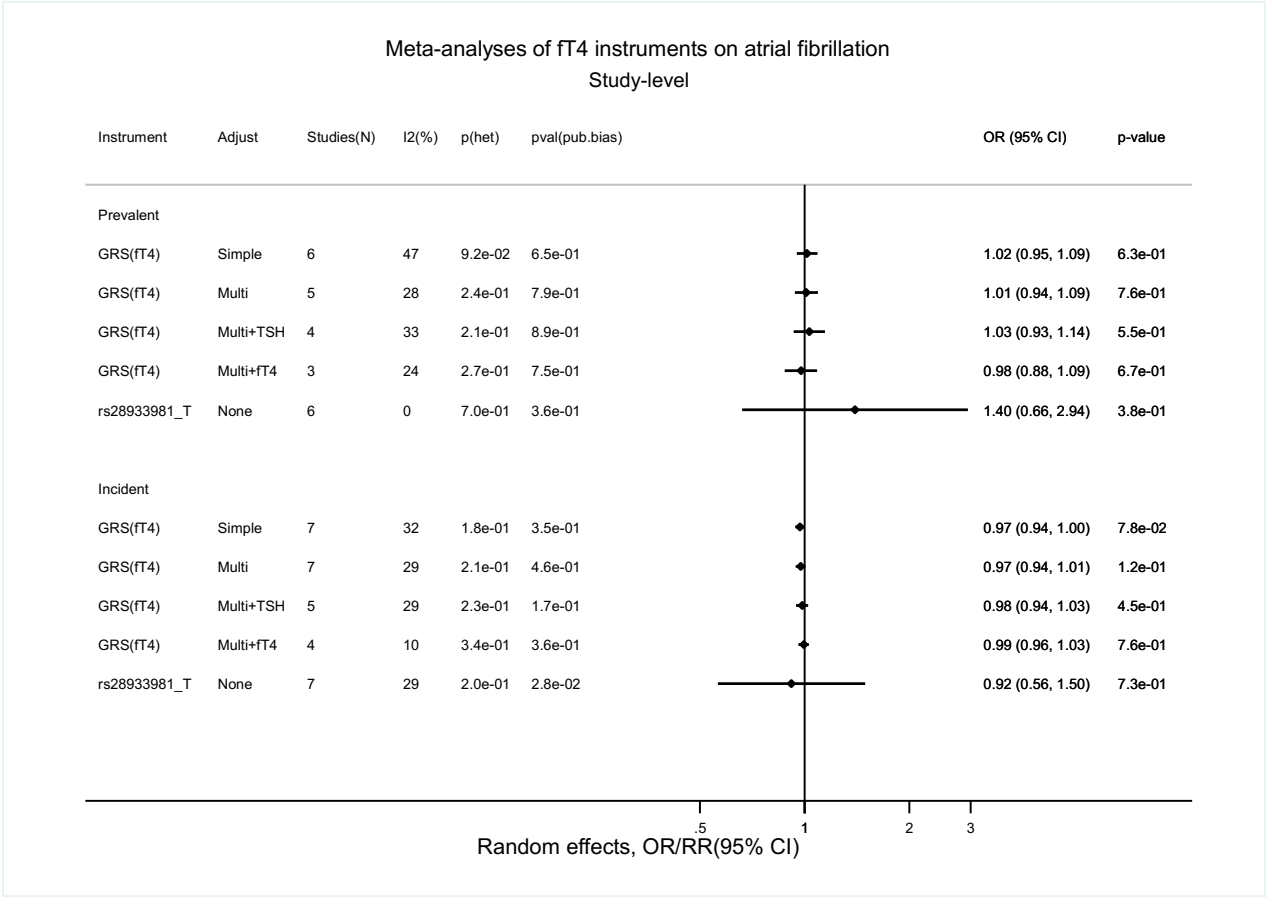


Figure 4A. Scatter plot of the TSH SNP effect on TSH (SD, mIU/L) (x-axis)

against the TSH SNP effect on atrial fibrillation (y-axis)

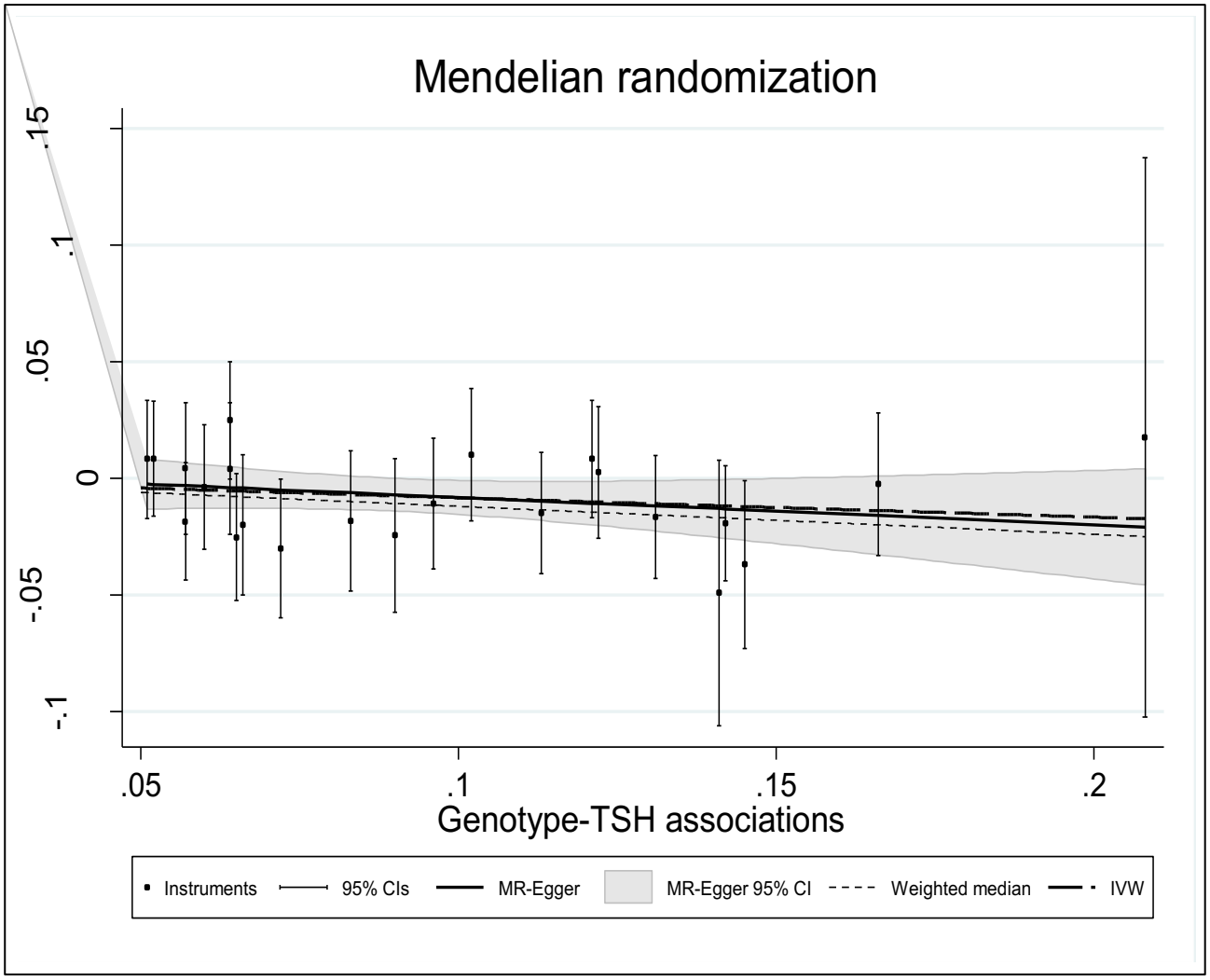
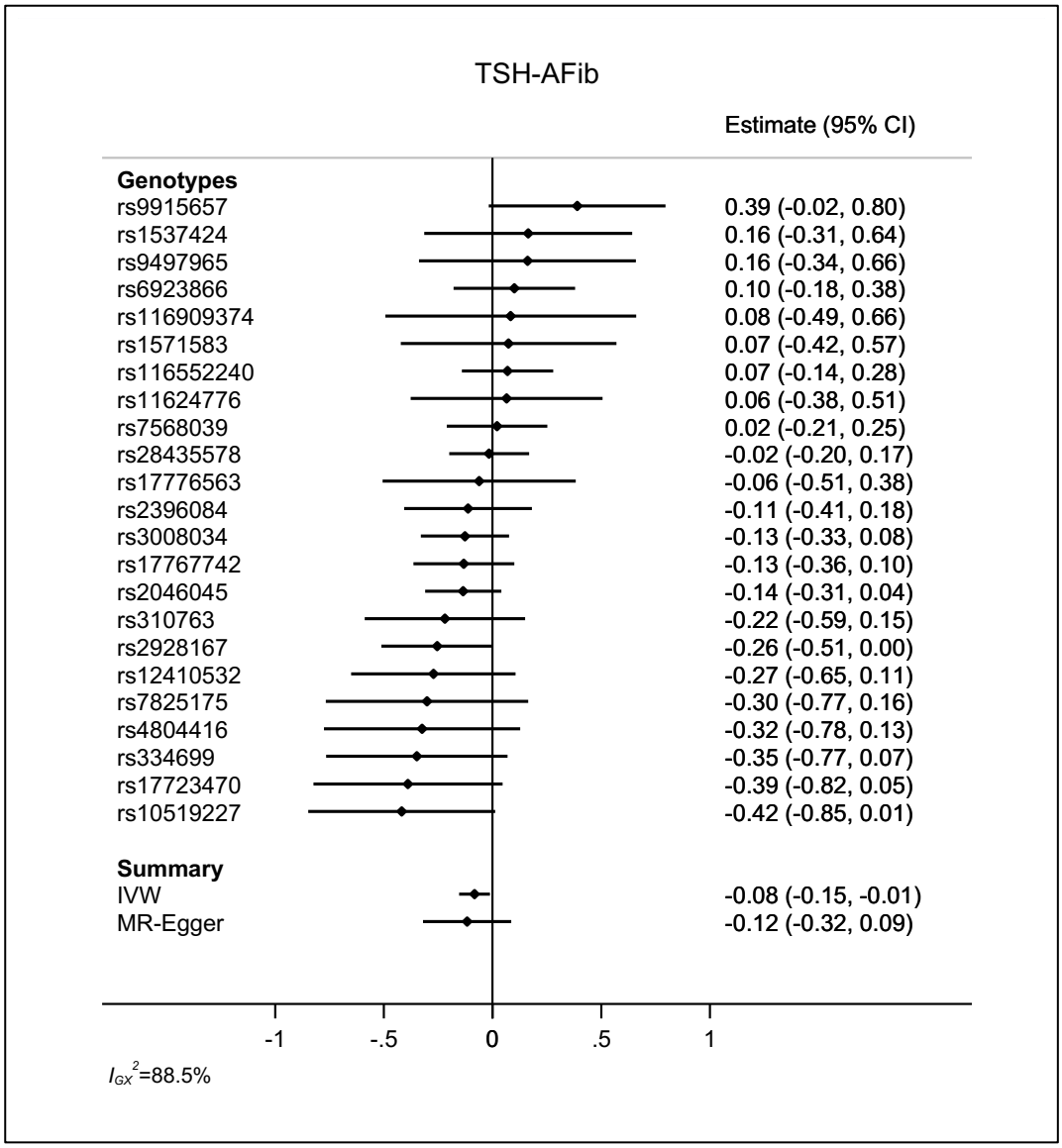


Figure 4B. Individual instrumental variable estimates for each of the 23 TSH-associated SNPs with risk of atrial fibrillation



Supplementary figures and tables

All supplementary files can be viewed in this shared google drive folder:

https://drive.google.com/open?id=136hwnwMlSmqhKIWGcjN1n7bT6SVcj9EN

Table 1. Baseline characteristics of study cohorts

	AGES	ARIC	CHS	FHS	LURIC	MESA	PREVEND	PROSPER	SHIP	VLAFR	WGHS
Total N	3153	9659	3770	2937	2559	2526	3515	5244	2186	680co/1058ca	19625
Age, years	76(5)	57(6)	76(5)	61(10)	63(11)	63(10)	49(12)	75(3)	53 (15)	56(14)	55(7)
Male, N(%)	1330(42)	4448(46)	1604(43)	1466(50)	1794(70)	1206(48)	1809(52)	2524(48)	1028(47)	459(68)	0(0)
Height, cm	167(9)	169(9)	164(10)	168(10)	170(9)	169(10)	174 (9)	165(9)	169(10)	173(11)	164(6)
Weight, kg	76(15)	78(16)	72 (14)	79(17)	80(14)	79(17)	79(14)	73(13)	80(16)	87 (18)	70(14)
BMI, kg/m²	27(4)	27(5)	27(5)	28(5)	28(4)	28(5)	26(4)	27(4)	28(5)	29(6)	26(5)
Current smoker, N(%)	400(13)	2028(21)	338(9)	458(16)	586(23)	287(11)	1235(35)	1392(27)	566(26)	128(19)	2211(11)
Systolic blood pressure, mmHg	143(20)	120(18)	135(21)	128(19)	141(24)	124(20)	129(20)	155(22)	132(19)		124(14)
Diastolic blood pressure, mmHg	74 (10)	71 (10)	70(12)	75(10)	81(12)	70(9)	74(10)	84(11)	82(11)		77(9)
Anti-hypertensive medication, N(%)	720(23)	2045(21)	1814(49)	927(32)	2220(87)	840(33)	416(14)	3882(74)	861(39)	458(67)	2469(13)
Diabetes, N(%)	363(12)	1104(11)	588(16)	322(11)	1510(41)	151(6)	133(4)	544(10)	257(12)	179(26)	469(2)
Prevalent coronary heart disease, N(%)	710(23)	560(6)	860(23)	289(10)	2018(79)	-	28(0.8)	708(14)	326(15)		-
Prevalent heart failure, N(%)	97(3)	359(4)	252(7)	42(1)	851(33)	-	6(0.2)	0(0)	276(13)	120(18)	-
Alcohol (>=2 drinks day), N(%)	27(0.9)	544(6)	271(7)	428(15)	902(35)	325(14)	0(0)	751(14)	189(9)	NA	806(4)
fT4 common Genetic Risk Score (GRS_{fT4})	0.38(0.15)	0.39(0.15)	3.07 (1.23)	0.37(0.13)	0.36(0.15)	0.37(0.15)	3.3(1.3)	0.28 (0.13)	0.39(0.15)	0.39(0.15)	3.25(1.28)
TTR rs28933981 risk allele (T), MAF%	0.43	0.29	0.17	0.65	0.26	0.10	0.40	NA	0.18	NA	0.24
fT4, ng/dL	NA	1.1(0.2)	1.2(0.3)	NA	1.4(0.3)	NA	1.0(0.2)	1.2(0.2)	1.1(0.2)	NA	1.1(0.2)
TSH, µIU/ml	NA	2.8(6.1)	3.0(4.9)	2.2(3.0)	1.8 (4.6)	NA	1.8 (3.6)	2.2 (2.0)	1.0(1.0)	NA	3.5(5.5)
Study years (baseline) (YYYY-YYYY)	2002-2004	1990-1992	1992-2009	1987-2012	1997-2000	2000-2002	1997-1998	1997-1999	2002 - 2006	1999-2005	1992-1995
Incident AF, N(%)	589(19)	1799(19)	1115(32)	368(13)	NA	137(5)	170(5)	505(10)	NA	NA	903
Years of follow-up for incident AF	baseline-2015	1990-2013	17	0-26	NA	10	1997-2010	3.5 yrs	NA	NA	18
Prevalent AF, N(%)	290(9)	0(0)	280(7)	107(4)	315(12)	NA	0(0)	0(0)	43(2)	1058	0(0)

Values correspond to n (%) or mean (standard deviation). Free T4 (fT4) gene score reflects a weighted sum of fT4 increasing alleles of 4 single-nucleotide polymorphisms (SNPs) (see Methods).

co/ca: controls/cases

The Age, Gene/Environment Susceptibility Study (AGES) Reykjavik study, the Atherosclerosis Risk in Communities (ARIC) study, the Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), The Ludwigshafen Risk and Cardiovascular Health (LURIC) study (LURIC), The Multi-Ethnic Study of Atherosclerosis (MESA), The Prevention of Renal and Vascular End-Stage Disease (PREVEND) cohort study, The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), the Study of Health in Pomerania (SHIP), the Vanderbilt-Lone-Atrial-Fibrillation-Registry (VLAFR), the Women's Genome Health Study (WGHS).

Table 2. Mendelian Randomization estimates of genetically predicted thyroid function on atrial fibrillation using publically available data on SNP-thyroid function and CHARGE AFIB consortium

Instrument SNP (G)	Exposure (X)	Outcome (Y)	MR method	N SNP	OR	95%CI	p-value	NOME* I^2_{GX},%	Pleiotropy, MR-Egger intercept, p	Heterogeneity Qstat, p	Between-instrument I^2_{MR}, %
TSH	TSH||SD(mIU/L)	AFIB	MR Egger	23	0.89	0.73-1.08	0.231	88	0.713	0.208	-
TSH	TSH||SD(mIU/L)	AFIB	Inverse variance weighted	23	0.92	0.86-0.98	0.015	-	-	0.247	16(0-49)
TSH	TSH||SD(mIU/L)	AFIB	Weighted median	23	0.89	0.81-0.97	0.009	-	-	-	-
fT4	fT4||SD(ng/dL)	AFIB	MR Egger	5	0.64	0.19-2.11	0.466	75	0.444	0.001	-
fT4	fT4||SD(ng/dL)	AFIB	Inverse variance weighted	5	1.08	0.82-1.43	0.594	-	-	0.0002	81(57-92)
fT4	fT4||SD(ng/dL)	AFIB	Weighted median	5	1.00	0.84-1.20	0.965	-	-	-	-
Hypothyroidism	Hypothyroidism	AFIB	MR Egger	4	1.16	0.64-2.09	0.634	55	0.607	0.060	-
Hypothyroidism	Hypothyroidism	AFIB	Inverse variance weighted	4	0.99	0.90-1.09	0.853	-	-	0.237	53(0-84)
Hypothyroidism	Hypothyroidism	AFIB	Weighted median	4	1.00	0.91-1.10	0.935	-	-	-	-
TPOAb	TPOAb levels||SD(IU/mL)	AFIB	MR Egger	4	0.35	0.004-34	0.649	0	0.766	0.960	-
TPOAb	TPOAb levels||SD(IU/mL)	AFIB	Inverse variance weighted	4	0.69	0.32-1.45	0.323	-	-	0.990	0(0-85)
TPOAb	TPOAb levels||SD(IU/mL)	AFIB	Weighted median	4	0.62	0.27-1.46	0.302	-	-	-	-

*NOME: "NO Measurement Error". TSH: Thyroid stimulating hormone. fT4: free thyroxine. TPOAb: Thyroid Peroxidase Antibody.

Summary of Project 1 and Project 2 conclusions

In this thesis, our aim was to evaluate causality for previous observational findings that hypothyroidism was associated with decreased kidney function and that hyperthyroidism was associated with increased risk of atrial fibrillation.

In both projects we used study-level data and summary-level data from previous GWAS. In both projects we used genetically different thyroid function instruments using common GWAS SNPs for hypothyroidism(32, 52), increased TSH within the reference range(29, 31), increased fT4 within the reference range(29, 31), and increased antibodies against thyroid peroxidase (TPOAb) (30, 53), in order to predict kidney function and atrial fibrillation. In project 1, we concluded that genetically predicted hypothyroidism is causally associated with decreased kidney function likely through an autoimmune mechanism; however, neither genetically predicted $eGFR_{crea}$ nor CKD is associated with thyroid function. In project 2, we concluded that genetically predicted increased TSH may have a causal role in decreased risk of AF, but genetic instruments for fT4, TPOAb, or hypothyroidism demonstrated weak instrument bias and pleiotropy.

Discussion and perspectives (strengths and limitations, future directions; 1 page)

Mendelian randomization is an epidemiological strategy with many designs and statistical approaches each with assumptions and limitations(1). In this thesis, we used several designs (study-level MR with and without mediation analysis, summary-level (also called two-sample MR), forward and bidirectional MR) and several statistical approaches (IVW, MR Egger, and weighted median) to quantify causal relationships and estimate directionality of these associations. Our findings that hypothyroidism is causally associated with decreased kidney function and that increased TSH was causally associated with decreased risk of AF were robust findings across different MR designs and different statistical approaches. However, the findings across the different thyroid instruments were not consistent; this could be an explanation for the biological pathways involved, but could also be due to weak instrument biases. We hypothesize that since instruments for hypothyroidism, which are mostly autoimmune(32), but not instruments for TSH(29, 31), was associated with decreased kidney function, that the mechanism may be autoimmune rather than related to TSH itself. Instruments for TSH but not fT4 were associated with AF; however, the fT4 instrument suffered from weak instrument bias. Furthermore, fT4 instruments were only instruments for fT4 in the reference range(29, 31), but no current instruments exist for fT4 outside the reference range. The strengths of the instruments in MR studies are dependent on the power of the previous GWAS. It is likely that the previous GWAS did not capture all instruments. In the future, larger GWAS are needed to find more instruments for thyroid function, this will increase the power of the MR studies. Furthermore, the MR assumes a single causal temporal pathway, but the hypothalamic-pituitary-thyroid-axis is a complex biological pathway with feedback loops, which make MR interpretations difficult with the current statistical methodologies (1).

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