Assessment of the Relationship Between Genetic Determinants of Thyroid Function and Atrial Fibrillation
A Mendelian Randomization Study

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IMPORTANCE Increased free thyroxine (FT_4) and decreased thyrotropin are associated with increased risk of atrial fibrillation (AF) in observational studies, but direct involvement is unclear.

OBJECTIVE To evaluate the potential direct involvement of thyroid traits on AF.

DESIGN, SETTING, AND PARTICIPANTS Study-level mendelian randomization (MR) included 11 studies, and summary-level MR included 55 114 AF cases and 482 295 referents, all of European ancestry.

EXPOSURES Genomewide significant variants were used as instruments for standardized FT_4 and thyrotropin levels within the reference range, standardized triiodothyronine (FT_3):FT_4 ratio, hypothyroidism, standardized thyroid peroxidase antibody levels, and hyperthyroidism. Mendelian randomization used genetic risk scores in study-level analysis or individual single-nucleotide polymorphisms in 2-sample MR for the summary-level data.

MAIN OUTCOMES AND MEASURES Prevalent and incident AF.

RESULTS The study-level analysis included 7679 individuals with AF and 49 233 referents (mean age [standard error], 62 [3] years; 15 859 men [29.7%]). In study-level random-effects meta-analysis, the pooled hazard ratio of FT_4 levels (nanograms per deciliter) for incident AF was 1.55 (95% CI, 1.09-2.20; P = .02; I^2 = 76%) and the pooled odds ratio (OR) for prevalent AF was 2.80 (95% CI, 1.41-5.54; P = .003; I^2 = 64%) in multivariable-adjusted analyses. The FT_4 genetic risk score was associated with an increase in FT_4 by 0.082 SD (standard error, 0.007; P < .001) but not with incident AF (risk ratio, 0.84; 95% CI, 0.62-1.14; P = .27) or prevalent AF (OR, 1.32; 95% CI, 0.64-2.73; P = .46). Similarly, in summary-level inverse-variance weighted random-effects MR, gene-based FT_4 within the reference range was not associated with AF (OR, 1.01; 95% CI, 0.89-1.14; P = .88). However, gene-based increased FT_3:FT_4 ratio, increased thyrotropin within the reference range, and hypothyroidism were associated with AF with inverse-variance weighted random-effects OR of 1.33 (95% CI, 1.08-1.63; P = .006), 0.88 (95% CI, 0.84-0.92; P < .001), and 0.94 (95% CI, 0.90-0.99; P = .009), respectively, and robust to tests of horizontal pleiotropy. However, the subset of hypothyroidism single-nucleotide polymorphisms involved in autoimmunity and thyroid peroxidase antibodies levels were not associated with AF. Gene-based hyperthyroidism was associated with AF with MR-Egger OR of 1.31 (95% CI, 1.05-1.63; P = .02) with evidence of horizontal pleiotropy (P = .045).

CONCLUSIONS AND RELEVANCE Genetically increased FT_3:FT_4 ratio and hyperthyroidism, but not FT_4 within the reference range, were associated with increased AF, and increased thyrotropin within the reference range and hypothyroidism were associated with decreased AF, supporting a pathway involving the pituitary-thyroid-cardiac axis.

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I n observational longitudinal studies, increased free thyroxine (FT_4) within the reference range,[1] subclinical hyperthyroidism,[2,3] and overt primary hyperthyroidism[2,4] are associated with increased risk of atrial fibrillation (AF), whereas overt and subclinical hypothyroidism are associated with reduced risk of AF.[2] Risk of AF is highest at the time of diagnosis of hyperthyroidism but risk persists despite antithyroid treatment.[4-7] This raises the question of whether FT_4 is on the causal pathway or instead a biomarker for the hyperthyroid-AF association.

Regulation of thyroid function is complex and involves not only the thyroid itself but also the pituitary and hypothalamus as well as feedback mechanisms. Produced and released by the pituitary, thyrotropin induces the thyroid to release thyroxine, which circulates in equilibrium between a sequestered, protein-bound form and the bioavailable free form, ie, FT_4. Free thyroxine levels are regulated by feedback onto the pituitary to suppress thyrotropin production. In thyroidal and peripheral tissues, FT_4 is converted to the active free triiodothyronine (FT_3) hormone, a process that can be assessed by the circulating FT_3:FT_4 ratio.[8] In the myocytes, FT_3 binds to nuclear receptors with positive inotropic (cardiac contractility) and chronotropic effects (heart rate and rhythm).[9] Thus, FT_4 levels are only 1 measure of thyroid function and its association with AF may not reflect a direct relationship even if there is causality along the pituitary-thyroid-cardiac axis.

The physiologic diversity of influences on thyroid function is reflected in the genes implicated by genome-wide association studies (GWAS) of FT_4 levels within the reference range,[10-12] FT_3:FT_4 ratio,[13] thyrotropin levels within the reference range,[10-12] hypothyroidism,[12,14,15] concentration of thyroid peroxidase antibodies[16,17] (TPOAb, a marker of autoimmune thyroid disease), and hyperthyroidism.[12] Genes implicated in FT_4 levels are involved in thyroid development, thyroid hormone action and transport, intracellular mechanisms of hormone action, and metabolism of thyroid hormones.[12]

The mendelian randomization (MR) instrumental variable design mimics a randomized clinical trial by leveraging allelic randomization during meiosis and subsequent irreversible exposure to genotype at conception and is typically less likely affected by confounding or reverse causation than conventional observational analyses.[18] If circulating FT_4 levels are directly involved in the development of AF, then genetic variation influencing FT_4 should also be associated with AF risk with effects that are quantitatively consistent with the observational associations.[19] Similar arguments may be made for other measures of thyroid function, such as circulating thyrotropin.

We used MR to explore whether FT_4 levels within the reference range, FT_3:FT_4 ratio, thyrotropin levels within the reference range, hypothyroidism, TPOAb, or hyperthyroidism may be on a causal pathway for AF. Mendelian randomization was implemented with genetic risk scores in study-level analysis from 11 studies with 56 912 participants and in a 2-sample strategy using summary-level analysis from 66 studies of 537 409 participants within the AF Genetics (AFGen) Consortium. To account for potential pleiotropy among instruments for the related traits of thyroid function, MR instruments for FT_4, thyrotropin, and hypothyroidism were stratified according to involvement in other thyroid traits.

Key Points

Question Are free thyroxine (FT_4) and thyrotropin levels within the reference range, triiodothyronine (FT_3):FT_4 ratio, hypothyroidism, thyroid peroxidase antibody levels, or hyperthyroidism on a direct pathway for atrial fibrillation (AF)?

Findings This mendelian randomization study of 55 114 individuals with AF and 482 295 referents found that genetically increased FT_3:FT_4 ratio and hyperthyroidism were associated with increased risk of AF, and thyrotropin within the reference range and hypothyroidism were inversely associated with risk of AF. There was no support for a direct involvement of FT_4 within the reference range or thyroid peroxidase antibody levels in AF.

Meaning Low thyrotropin, as an early sign of an overactive thyroid gland, with a concomitant increased FT_3:FT_4 ratio are genetically associated with AF.
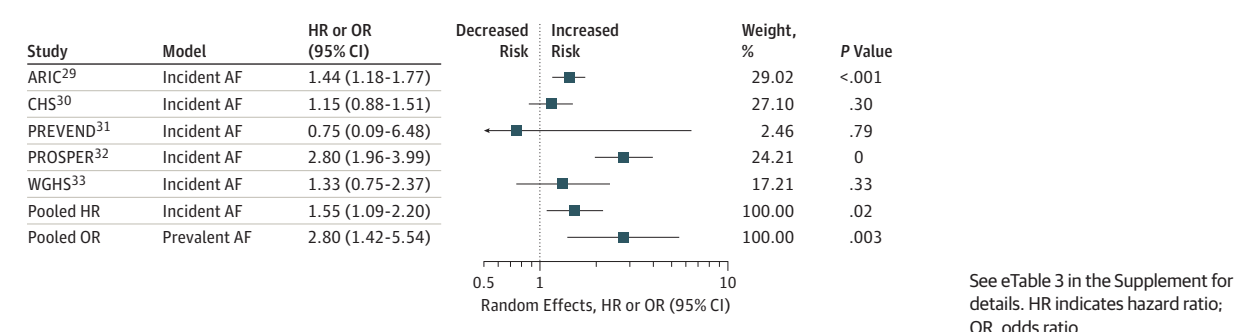
Methods

The analytic approach consisted of 2 parts. First, we assessed the relevance of FT_4 levels in AF by performing MR with a genetic risk score of 4 FT_4-associated single-nucleotide polymorphisms (SNPs) among population-based cohorts and population-based randomized clinical trials. Second, we assessed potential relevance of an expanded set of thyroid instruments for FT_4 and thyrotropin levels within the reference range, FT_3:FT_4 ratio, hypothyroidism, TPOAb, and hyperthyroidism (determined by low thyrotropin levels) by performing an MR through the 2-sample method within published summary-level GWAS data for thyroid traits[10,11,13-17,20] and AF[21,22] (eTable 1 in the Supplement). See the eMethods in the Supplement for study information, study-specific funding and disclosure information, genetic instruments and genotyping, and study-specific characteristics of genotyping methodology.

Participants

A total of 56 912 participants with 7679 AF cases (2093 prevalent and 5586 incident) and 49 233 referents (eTable 2 in the Supplement) were included from 11 cross-sectional and prospective cohort studies of European ancestry contributing to the AFGen Consortium. Mean age ranged from 53 to 76 years. Among the 11 studies, FT_4 (nanograms per deciliter) and thyrotropin (milli-international units per liter) levels were available respectively in 7 studies (n = 26 089) and 8 studies (n = 27 916). All protocols were approved by local institutional review boards. Participant consent was obtained if the local institutional review boards required this. Analyses were not restricted to thyroid measures within the reference range.

Summary-Level Analysis

Instrument-exposure associations (ie, FT_4, thyrotropin, FT_3:FT_4 ratio, hypothyroidism, TPOAb, and hyperthyroidism) were

Figure 1. Observational Meta-analyses of Free Thyroxine (FT_4) on Atrial Fibrillation (AF) (Study Level)

Study	Model	HR or OR (95% CI)	Decreased Risk / Increased Risk	Weight, %	P Value
ARIC[29]	Incident AF	1.44 (1.18-1.77)		29.02	<.001
CHS[30]	Incident AF	1.15 (0.88-1.51)		27.10	.30
PREVEND[31]	Incident AF	0.75 (0.09-6.48)		2.46	.79
PROSPER[32]	Incident AF	2.80 (1.96-3.99)		24.21	0
WGHS[33]	Incident AF	1.33 (0.75-2.37)		17.21	.33
Pooled HR	Incident AF	1.55 (1.09-2.20)		100.00	.02
Pooled OR	Prevalent AF	2.80 (1.42-5.54)		100.00	.003

Random Effects, HR or OR (95% CI)

See eTable 3 in the Supplement for details. HR indicates hazard ratio; OR, odds ratio.

from GWAS for various thyroid traits among individuals of European ancestry.[12-17] The instrument-AF associations for common variants (minor allele frequency, >1%) came from a GWAS of AF from the AFGen Consortium among individuals of European ancestry, including 66 studies with a total of 55 114 individuals with AF (7672 incident, 47442 prevalent) and 482 295 referents.[21,22] In the AFGen GWAS, we identified 30 of 31 SNPs for FT_4 within the reference range,[12] 1 of 1 SNP for FT_3:FT_4 ratio (the *DIO1*, rs2235544),[13] 56 of 61 SNPs for thyrotropin within the reference range,[12] 15 of 30 SNPs for hypothyroidism,[14,23] 4 of 5 SNPs for TPOAb concentration,[16,17] and 8 of 8 SNPs for hyperthyroidism (which was defined by low thyrotropin levels in population-based cohorts in which patients receiving medication for autoimmune thyroiditis were excluded).[12] For the rare variant (minor allele frequency, 0.4%) in the *TTR* gene, the association with AF came from an exome-wide association study by meta-analysis including 22 346 cases and 132 086 referents.[21]

Atrial fibrillation diagnosis used cohort-specific definitions, including physician adjudication, questionnaire self-report, electrocardiography, and diagnosis codes for AF or flutter (*International Classification of Diseases, Ninth Revision*: 427.3, 427.31 or 427.32; *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*: I48) present in hospitalization discharges or death certificates as previously described.[22] For analyses of incident AF, individuals with baseline AF were excluded.

Instrument strength in MR was estimated with an approximated F statistic[24] (eMethods in the Supplement). The instruments for FT_4 in study-level analyses all had instrument strength above the minimum required threshold of 10 ($F > 10$), except for 1 SNP (*AADAT*, rs7694879). The genetic instruments for summary-level analyses all had instrument strengths (F) larger than 10 (ie, for FT_4, range: 29-394; FT_3:FT_4 ratio, 21; thyrotropin, 29-576; hypothyroidism, 23-105; TPOAb, 10-19; and hyperthyroidism, 30-94). Mendelian randomization results are presented in SD units of the biomarkers (eMethods in the Supplement). We performed associations for 6 primary hypotheses (FT_4, FT_3:FT_4 ratio, thyrotropin, hypothyroidism, TPOAb, and hyperthyroidism). While tests of these primary hypotheses involved multiple secondary analyses, the tests were correlated, we therefore considered a 2-tailed $P < .008$ (= .05/6) to be significant.

Study-Level MR Using a Genetic Risk Score

Instrumental estimates were combined across studies using inverse-variance weighted random-effects (IVW-RE) meta-analyses. First, we examined the observational associations of FT_4 or thyrotropin with prevalent or incident AF using logistic or Cox regression, respectively. A genetic risk score for FT_4 levels (GRS_{FT_4}) was created by summing the allele count of 4 genotyped alleles (*DIO1, LHX3, AADAT,* and *FOXE1*) or the maximum likelihood dose of imputed alleles at each of the variants, weighted by the effect size, ie, the β coefficients. Mendelian randomization instrumental effects were derived by the instrumental variable ratio (Wald) estimator, which was the ratio of the meta-analysis β coefficient from the genetic risk score on AF association divided by the meta-analysis β coefficient from the genetic risk score on FT_4 association, with standard errors calculated using the δ method.[25]

Two-Sample Summary-Level MR

For the individual SNPs, MR instrumental estimates were determined by instrumental variable ratio. To determine the primary overall instrumental estimate, IVW fixed-effects MR was applied across the individual instrumental estimates and their standard errors, assuming all genetic variants are valid instruments with no pleiotropy.[26] The between-instrument heterogeneity Cochran Q statistic and the I^2_{MR} index were used to assess heterogeneity in the MR.[27] By several approaches, we performed sensitivity analyses to investigate potential pleiotropic bias[26,28]: IVW-RE MR, MR-Egger regression, weighted median MR, funnel plots, and leave-1-variant-out analysis for IVW-RE, where 1 variant at a time is left out.

Results

FT_4 in Study-Level Analysis

Characteristics of studies are presented in eTable 2 in the Supplement. In meta-analysis of multivariable-adjusted, observational study-level data, the pooled hazard ratio of FT_4 (nanograms per deciliter; to convert to picomoles per liter, multiply by 12.871) for incident AF was 1.55 (95% CI, 1.09-2.20; $P = .02$; $I^2 = 76\%$) with no evidence of participation bias (P for Egger = .94) and the pooled odds ratio (OR) for prevalent AF was 2.80 (95% CI, 1.42-5.54; $P = .003$; $I^2 = 64\%$) with evidence of participation bias (P for Egger = .003) (**Figure 1** and

Table. Two-Sample Mendelian Randomization Estimates of Relationship Between Genetically Predicted Thyroid Function and Combined Incident and Prevalent Atrial Fibrillation Using CHARGE AF Genetics Consortium in European Individuals[a]

Instrument	Exposure	MR Method	No. of SNP	OR (95% CI)	P Value	Heterogeneity	
						I^2_{MR} % (95% CI)	P Value
FT_4	FT_4	IVW-FE	30	1.00 (0.95-1.05)	.93	81 (73-86)	<.001
	FT_4	IVW-RE	30	1.01 (0.89-1.14)	.88	81 (73-86)	<.001
DIO1	FT_3:FT_4 ratio	IVR	1	1.33 (1.08-1.63)	<.001	NA	NA
Thyrotropin	Thyrotropin	IVW-FE	56	0.88 (0.89-0.91)	<.001	27 (0-48)	.04
	Thyrotropin	IVW-RE	56	0.88 (0.84-0.92)	<.001	27 (0-48)	.04
Hypothyroidism	Hypothyroidism	IVW-FE	15	0.95 (0.91-0.97)	<.001	52 (13-73)	.01
	Hypothyroidism	IVW-RE	15	0.94 (0.90-0.99)	<.001	52 (13-73)	.01
TPOAb	TPOAb levels	IVW-FE	4	0.76 (0.49-1.18)	.22	0 (0-85)	.43
	TPOAb levels	IVW-RE	4	0.76 (0.49-1.18)	.22	0 (0-85)	.43
Hyperthyroidism	Hyperthyroidism	IVW-FE	8	1.05 (1.03-1.08)	<.001	77 (54-88)	<.001
	Hyperthyroidism	IVW-RE	8	1.04 (0.99-1.10)	.13	77 (54-88)	<.001

Abbreviations: AF, atrial fibrillation; FT_4, free thyroxine; FT_3, free triiodothyronine; GWAS, genome-wide association studies; IVR, instrumental variable ratio (Wald) estimator; IVW-FE, inverse-variance weighted fixed-effects MR; IVW-RE, inverse-variance weighted random-effects MR; MR, mendelian randomization; NA, not applicable; OR, odds ratio; SNP, single-nucleotide polymorphism; TPOAb, thyroid peroxidase antibody levels.

[a] From GWAS of 66 studies with 55 114 AF cases and 482 295 referents.

eTable 3 in the Supplement).[29-33] Meanwhile, each increment in the GRS$_{FT4}$ was associated with a 0.082 SD (standard error, 0.007) increase of standardized FT$_4$ ($P < .001$) in multivariable-adjusted random-effects meta-analyses (eTables 4 and 5 in the Supplement) with only minimal potential pleiotropic effects on thyrotropin in multivariable-adjusted random-effects meta-analyses (eTable 6 in the Supplement). In the MR analysis, the instrumental estimates of FT$_4$ (per SD) were nonsignificant for incident or prevalent AF with an overall risk ratio of 0.84 (95% CI, 0.62-1.14; $P = .27$) across 7 studies and OR of 1.32 (95% CI, 0.64-2.73, $P = .46$) across 5 studies in multivariable-adjusted random-effects meta-analyses, respectively (eTables 7 and 8 in the Supplement). Results were similar if analyses were restricted to studies that had complete information on GRS$_{FT4}$, FT$_4$ and AF (eTable 9 in the Supplement), if the *TTR* variant was included in the GRS$_{FT4}$ (eTables 7 and 8 in the Supplement), or if the *TTR* variant was analyzed alone (eTables 7, 8, and 10 in the Supplement).

FT_4 in Summary-Level Analysis

The individual instrument-exposure and instrument-outcome associations are shown in eTable 11 in the Supplement. In summary-level MR analysis, the IVW-RE OR for combined prevalent and incident AF per SD of FT$_4$ within the reference range was 1.01 (95% CI, 0.89-1.14; $P = .88$) (Table and eFigure 1A in the Supplement). Results were similar for prevalent and incident AF separately in summary-level IVW fixed-effects and MR-Egger analysis (eTables 12, 13, and 14 in the Supplement). Weighted median analysis was significant with OR for AF per SD of FT$_4$ of 0.87 (95% CI, 0.79-0.95; $P = .001$) (eTable 12 in the Supplement).

Estimates for the individual FT$_4$ instruments in summary-level analysis were not homogeneous (Figure 2), which was reflected in the I^2_{MR} values of 81% (95% CI, 73%-86%; $P < .001$) (Table). There was no difference in the point estimate when parsing out the FT$_4$ SNPs into those associated with thyrotropin (IVW-RE OR, 1.00; 95% CI, 0.78-1.46; $P = .68$) and those

not (IVW-RE OR, 1.00; 95% CI, 0.87-1.14; $P = .96$) (P for interaction = 0.70) (Figure 2). FT$_4$ SNPs were distributed symmetrically about the combined effect size in the funnel plot (eFigure 1B in the Supplement), and MR-Egger did not show evidence of horizontal pleiotropy (P for MR-Egger = .63) (eTable 12 in the Supplement). Leave-1-variant-out analysis did not identify variants with exaggerated influence on the combined effect estimate (eTable 15 in the Supplement). Subanalysis of identical instruments (n = 4) as in the study-level analysis revealed a similar point estimate (eTable 16 in the Supplement).
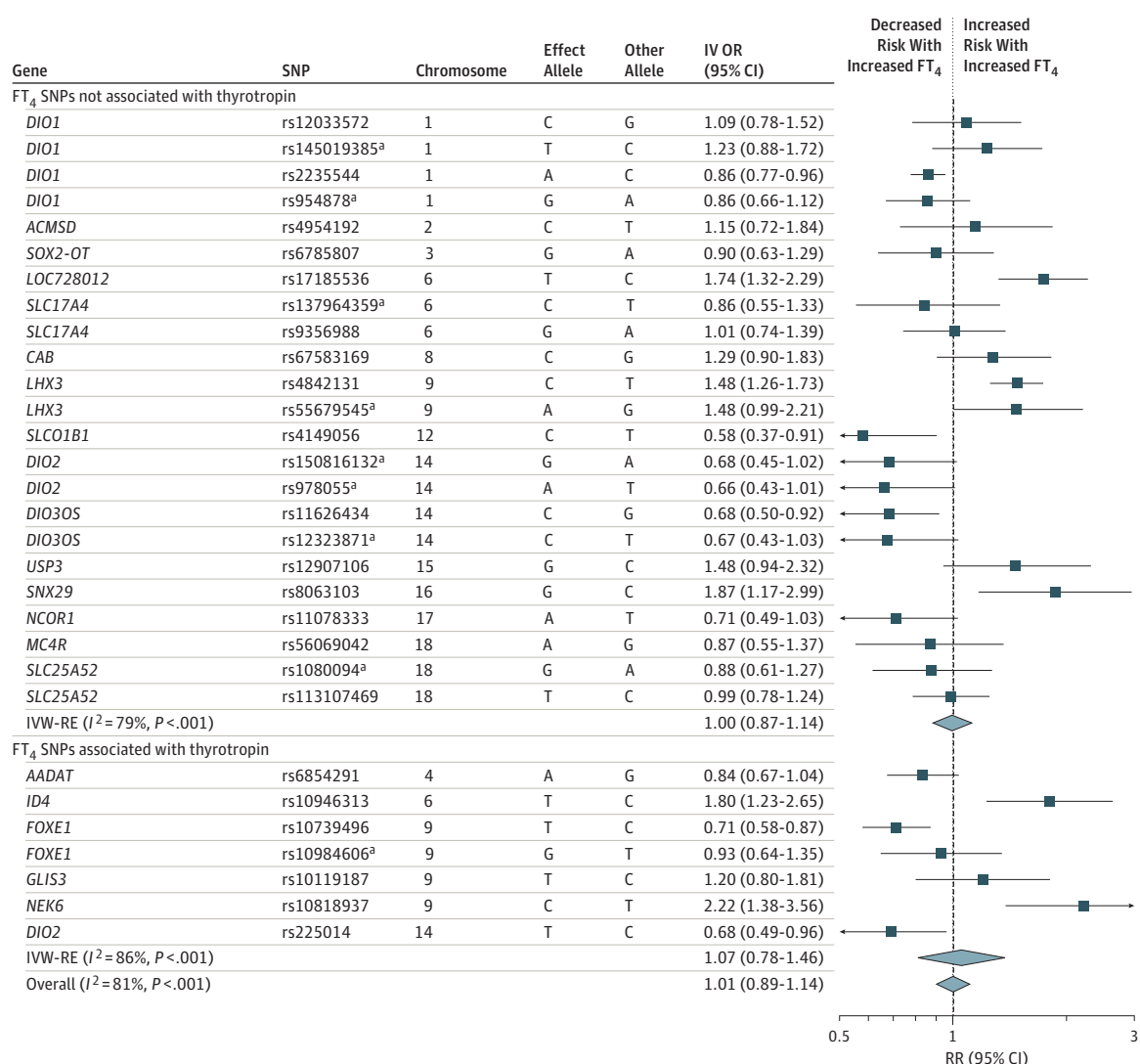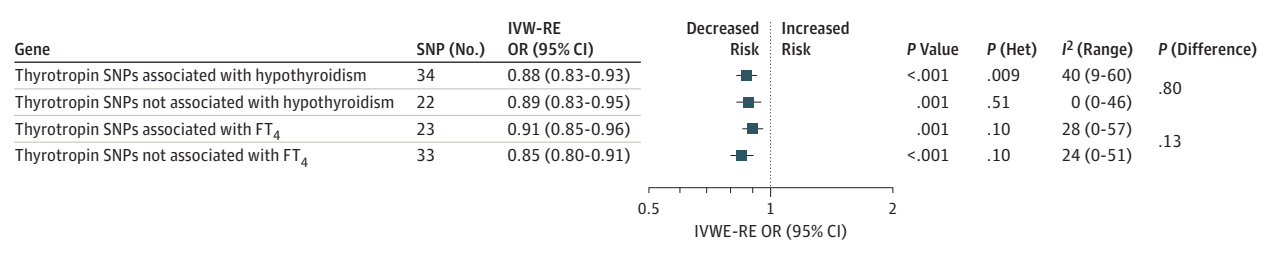
FT_3:FT_4 Ratio

In 2-sample summary-level MR, a genetically predicted 1 SD-increase in FT$_3$:FT$_4$ ratio by the *C* allele was associated with increased AF with an OR of 1.33 (95% CI, 1.08-1.63; $P = .006$) (Table and eTable 12 in the Supplement). Results were similar for incident and prevalent AF separately (eTables 13 and 14 in the Supplement).

Thyrotropin

In random-effects meta-analysis of observational study-level data, the pooled hazard ratio of thyrotropin (milli-international units per liter) for incident AF was 1.00 (95% CI, 0.98-1.01; $P = .87$; $I^2 = 23\%$) with $P = .06$ for participation bias, and the pooled OR for prevalent AF was 0.99 (95% CI, 0.97-1.02; $P = .52$; $I^2 = 0\%$) with no evidence of participation bias ($P = .52$) in multivariable-adjusted analyses (eTable 17 in the Supplement).

Instrument-exposure and instrument-outcome associations are shown in eTable 18 in the Supplement. A genetically predicted 1 SD-increase in thyrotropin was inversely associated with AF with IVW-RE OR of 0.88 (95% CI, 0.84-0.92; $P < .001$; $I^2_{MR} = 27\%$) (Table and eFigure 2A and B in the Supplement). Results were similar with the MR-Egger and weighted median analyses (eTable 12 in the Supplement). The thyrotropin variants were distributed symmetrically about the

Figure 2. Summary-Level Analysis of FT_4 SNPs and Atrial Fibrillation

Gene	SNP	Chromosome	Effect Allele	Other Allele	IV OR (95% CI)	Decreased Risk With Increased FT_4 / Increased Risk With Increased FT_4
FT_4 SNPs not associated with thyrotropin						
DIO1	rs12033572	1	C	G	1.09 (0.78-1.52)	
DIO1	rs145019385[a]	1	T	C	1.23 (0.88-1.72)	
DIO1	rs2235544	1	A	C	0.86 (0.77-0.96)	
DIO1	rs954878[a]	1	G	A	0.86 (0.66-1.12)	
ACMSD	rs4954192	2	C	T	1.15 (0.72-1.84)	
SOX2-OT	rs6785807	3	G	A	0.90 (0.63-1.29)	
LOC728012	rs17185536	6	T	C	1.74 (1.32-2.29)	
SLC17A4	rs137964359[a]	6	C	T	0.86 (0.55-1.33)	
SLC17A4	rs9356988	6	G	A	1.01 (0.74-1.39)	
CAB	rs67583169	8	C	G	1.29 (0.90-1.83)	
LHX3	rs4842131	9	C	T	1.48 (1.26-1.73)	
LHX3	rs55679545[a]	9	A	G	1.48 (0.99-2.21)	
SLCO1B1	rs4149056	12	C	T	0.58 (0.37-0.91)	
DIO2	rs150816132[a]	14	G	A	0.68 (0.45-1.02)	
DIO2	rs978055[a]	14	A	T	0.66 (0.43-1.01)	
DIO3OS	rs11626434	14	C	G	0.68 (0.50-0.92)	
DIO3OS	rs12323871[a]	14	C	T	0.67 (0.43-1.03)	
USP3	rs12907106	15	G	C	1.48 (0.94-2.32)	
SNX29	rs8063103	16	G	C	1.87 (1.17-2.99)	
NCOR1	rs11078333	17	A	T	0.71 (0.49-1.03)	
MC4R	rs56069042	18	A	G	0.87 (0.55-1.37)	
SLC25A52	rs1080094[a]	18	G	A	0.88 (0.61-1.27)	
SLC25A52	rs113107469	18	T	C	0.99 (0.78-1.24)	
IVW-RE (I^2 = 79%, P <.001)					1.00 (0.87-1.14)	
FT_4 SNPs associated with thyrotropin						
AADAT	rs6854291	4	A	G	0.84 (0.67-1.04)	
ID4	rs10946313	6	T	C	1.80 (1.23-2.65)	
FOXE1	rs10739496	9	T	C	0.71 (0.58-0.87)	
FOXE1	rs10984606[a]	9	G	T	0.93 (0.64-1.35)	
GLIS3	rs10119187	9	T	C	1.20 (0.80-1.81)	
NEK6	rs10818937	9	C	T	2.22 (1.38-3.56)	
DIO2	rs225014	14	T	C	0.68 (0.49-0.96)	
IVW-RE (I^2 = 86%, P <.001)					1.07 (0.78-1.46)	
Overall (I^2 = 81%, P <.001)					1.01 (0.89-1.14)	

RR (95% CI) scale: 0.5 — 1 — 3

The mendelian randomization effect estimate is risk of atrial fibrillation (IV OR) per standardized FT_4.

[a] Independent SNPs within a locus as defined by Teumer et al.[12]

FT_4 indicates free thyroxine; IV OR, instrumental variable odds ratio; IVW-RE, inverse variance-weighted random-effects; RR, risk ratio; SNP, single-nucleotide polymorphism.

combined effect size in the funnel plot (eFigure 2C in the Supplement), and MR-Egger did not show evidence of horizontal pleiotropy (P for MR-Egger = .95) (eTable 12 in the Supplement). Leave-1-variant-out analysis did not identify variants with exaggerated influence on the combined effect estimate (eTable 19 in the Supplement). Results were similar for incident and prevalent AF separately (eTables 13 and 14 in the Supplement). The IVW-RE association of thyrotropin levels with AF was stronger among thyrotropin SNPs that were also identified in GWAS for hypothyroidism vs those that were not and was stronger among thyrotropin SNPs that were not associated with FT_4 vs those that were, but none of these differences was statistically significant (**Figure 3**).

Hypothyroidism and TPOAb

Genetically predicted hypothyroidism was inversely associated with AF with an IVW-RE OR of 0.94 (95% CI, 0.90-0.99; P = .009; I^2_{MR} = 52%) (Table and eFigure 3A, eTable 12, eTable 20 in the Supplement). This association was significantly stronger among hypothyroid SNPs that were also identified for association with thyrotropin (OR, 0.90; 95% CI, 0.86-0.94) than those that were also identified with autoimmune function (OR, 0.99; 95% CI, 0.94-1.04; P for difference between effects of autoimmune SNPs vs thyrotropin SNPs = .006) (**Figure 4**). Results were similar for prevalent AF (eTable 14 in the Supplement) and in the same direction but not significant for incident AF (eTable 13 in the Supplement). Leave-1-variant-out analysis did not
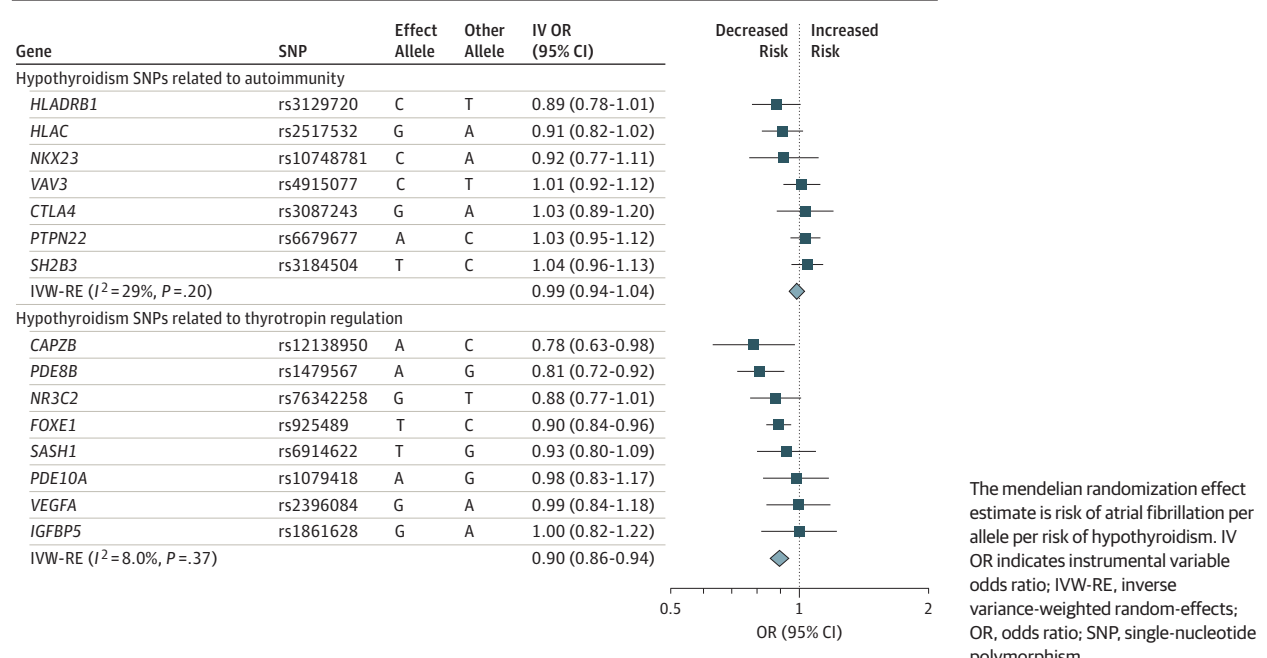
Figure 3. Summary-Level Analysis of SNP (Thyrotropin) and Atrial Fibrillation

Gene	SNP (No.)	IVW-RE OR (95% CI)	Decreased Risk / Increased Risk	P Value	P (Het)	I^2 (Range)	P (Difference)
Thyrotropin SNPs associated with hypothyroidism	34	0.88 (0.83-0.93)		<.001	.009	40 (9-60)	.80
Thyrotropin SNPs not associated with hypothyroidism	22	0.89 (0.83-0.95)		.001	.51	0 (0-46)	
Thyrotropin SNPs associated with FT$_4$	23	0.91 (0.85-0.96)		.001	.10	28 (0-57)	.13
Thyrotropin SNPs not associated with FT$_4$	33	0.85 (0.80-0.91)		<.001	.10	24 (0-51)	

0.5   1   2
IVWE-RE OR (95% CI)

The mendelian randomization effect estimate is risk of atrial fibrillation per standardized thyrotropin. FT$_4$ indicates free thyroxine; het, heterogeneity; IV OR, instrumental variable odds ratio; IVW-RE, inverse variance-weighted random-effects; OR, odds ratio; SNP, single-nucleotide polymorphism.

Figure 4. Summary-Level Analysis of SNP (Hypothyroidism) and Atrial Fibrillation

Gene	SNP	Effect Allele	Other Allele	IV OR (95% CI)	Decreased Risk / Increased Risk
Hypothyroidism SNPs related to autoimmunity					
HLADRB1	rs3129720	C	T	0.89 (0.78-1.01)	
HLAC	rs2517532	G	A	0.91 (0.82-1.02)	
NKX23	rs10748781	C	A	0.92 (0.77-1.11)	
VAV3	rs4915077	C	T	1.01 (0.92-1.12)	
CTLA4	rs3087243	G	A	1.03 (0.89-1.20)	
PTPN22	rs6679677	A	C	1.03 (0.95-1.12)	
SH2B3	rs3184504	T	C	1.04 (0.96-1.13)	
IVW-RE (I^2 =29%, P =.20)				0.99 (0.94-1.04)	
Hypothyroidism SNPs related to thyrotropin regulation					
CAPZB	rs12138950	A	C	0.78 (0.63-0.98)	
PDE8B	rs1479567	A	G	0.81 (0.72-0.92)	
NR3C2	rs76342258	G	T	0.88 (0.77-1.01)	
FOXE1	rs925489	T	C	0.90 (0.84-0.96)	
SASH1	rs6914622	T	G	0.93 (0.80-1.09)	
PDE10A	rs1079418	A	G	0.98 (0.83-1.17)	
VEGFA	rs2396084	G	A	0.99 (0.84-1.18)	
IGFBP5	rs1861628	G	A	1.00 (0.82-1.22)	
IVW-RE (I^2 =8.0%, P =.37)				0.90 (0.86-0.94)	

0.5   1   2
OR (95% CI)

The mendelian randomization effect estimate is risk of atrial fibrillation per allele per risk of hypothyroidism. IV OR indicates instrumental variable odds ratio; IVW-RE, inverse variance-weighted random-effects; OR, odds ratio; SNP, single-nucleotide polymorphism.

identify variants with exaggerated influence on the combined effect estimate (eTable 21 in the Supplement). Genetically predicted 1 SD–increase in TPOAb was not associated with AF in the MR analyses (eTable 12, eTable 20, and eFigure 4 in the Supplement).

Hyperthyroidism

Genetically predicted hyperthyroidism was associated with AF with an IVW-RE OR of 1.04 (95% CI, 0.99-1.10; P = .13; I^2_{MR} = 77%) (Table and eTable 12, eTable 22, eFigure 5A in the Supplement) with similar but significant results in IVW fixed-effects and weighted median analysis. Funnel plot (eFigure 5B in the Supplement) and MR-Egger showed evidence of pleiotropy (P for MR-Egger = .045), and the MR-Egger OR was 1.31 (95% CI, 1.05-1.63; P = .02). Results were similar for incident and prevalent AF (eTables 13 and 14 in the Supplement). Leave-1-variant-out analysis did not identify variants with exaggerated influence on the combined effect estimate (eTable 23 in the Supplement).

Discussion

In this MR study, the combined genetic association of FT$_4$ instruments did not support a direct effect of FT$_4$ levels in the reference range on AF. However, the pituitary-thyroid-cardiomyocyte axis was implicated through the significant gene-based effects on AF of FT$_3$:FT$_4$ ratio, hyperthyroidism, thyrotropin, and hypothyroidism, the latter largely limited to instruments with effects on thyrotropin as opposed to autoimmune function. The result diverges sharply from observational associations of FT$_4$ with AF, although those associations may have been influenced by inclusion of individuals with overt or subclinical thyroid disease,[1-3] an explanation that would be supported by the gene-based findings here for hypothyroidism, hyperthyroidism, and thyrotropin.

The hypothalamic-pituitary-thyroid-cardiac axis represents a series of phenotypes that are dependent on phenotypes upstream the axis as well as downstream through nega-

tive feedback mechanisms. This vertical pleiotropy was reflected by overlap of some of the instruments between some pairs of thyroid traits and by subgenomewide significant association of instruments with a second thyroid trait. By stratifying the MR analysis according to unique vs shared instruments, we endeavored to parse out influences on AF along the axis. These analyses supported the idea that gene-based effects on AF of thyrotropin, including those associated with thyroid disease, were qualitatively different from those of FT_4 within the normal range.

However, a larger question is why, in spite of very strong instruments, gene-based effects of FT_4 were largely null compared with significant associations upstream of FT_4 represented by thyrotropin? Some answers may be found by contrasting the instrumental associations with thyroid measures conditional on thyroid status. The FT_4 SNPs derive from associations among individuals with FT_4 levels within the reference range and no evidence of thyroid disease.[10-12] Further, when these SNPs were combined into a genetic risk score, they were not associated with thyroid disease in the forms of Graves disease, hypothyroidism, or hyperthyroidism.[12] By contrast, the instruments for thyrotropin within the reference range were associated with hypothyroidism and hyperthyroidism.[12] These distinctions may emphasize a diseased thyroid state rather than normal variation in thyroid function as driving the instrumental associations with AF. Such an interpretation is consistent with variability in observational associations between thyrotropin and AF, where the proportion of individuals with subclinical or overt thyroid disease may not have been adequately controlled.[2] Notably, the MR results for thyrotropin in this report are consistent with a large Danish observational study finding (N = 586 460) that hyperthyroidism and hypothyroidism were associated with increased and decreased risk of AF, respectively, and that there was an inverse linear association between thyrotropin and risk of AF in a sample that included individuals with thyrotropin outside of the normal range.[2]

Similarly, significant instrumental associations of FT_3:FT_4 ratio downstream of FT_4 need to be reconciled with the null result for FT_4. Here, we suggest that conversion of FT_4 to FT_3 (and therefore the value of the FT_3:FT_4 ratio) may be sufficiently regulated to limit the extent to which variation of FT_4 within the normal range may be propagated downstream. Teumer et al[12] examined this relationship for 3 independent genomewide significant SNPs for FT_4 levels mapping to 2 genes, *AADAT* (rs6854291) and *SLC17A4*, (9356988, rs137964359).

While the SNP at *AADAT* was associated with FT_3 and FT_3:FT_4 ratio, the 2 SNPs at *SLC17A4* were null suggesting at least partially independent control of FT_3 with respect to FT_4. Incidentally, the MR association of variation in the deiodinase (*DIO1*, rs2235544) may recommend pharmacologic inhibition of deiodinase-1 to block conversion of FT_4 to FT_3 in therapeutic protection from AF, although such treatment could potentially put patients at risk for other diseases as deiodinase-1 is expressed in many tissues.[34]

Limitations

The study has several limitations. Structurally, samples contributing to the study-level MR were also included in the much larger AF GWAS used for the summary-level MR, implying some dependence in the concordant results, even as the methodologies were different. The European ancestry of the samples also limits generalizability to other ancestries. In addition to the vertical pleiotropy considered above, there was evidence for horizontal pleiotropy that was addressed through current best practices for MR sensitivity analysis,[19] all of which supported the primary conclusions. However, as with all MR studies, we could not address unobserved pleiotropy. Given the divergence of the observational and MR instrumental findings for FT_4, there remains a need for more analysis, especially downstream of FT_4 along the hypothalamic-pituitary-thyroid-cardiac axis. Our ability to draw mechanistic inferences downstream of FT_4 was limited by the availability of only 1 instrument for the FT_3:FT_4 ratio and the lack of any instruments for FT_3, although this hormone, as opposed to FT_4, is transported into cardiomyocytes and thought to be more biologically relevant.[9]

Conclusions

In conclusion, we demonstrated that genetically increased FT_3:FT_4 ratio and hyperthyroidism are associated with increased AF with inverse associations for genetically predicted hypothyroidism and increased thyrotropin supporting relevance of the pituitary-thyroid-cardiac axis in AF susceptibility. However, genetically increased FT_4 within the reference range was not associated with AF, and the current lack of additional genetic instruments, particularly for FT_3, makes it difficult to infer a specific causal agent in the link between thyroid function and AF.

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