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Clinical Research Article

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Genetically Determined TSH Level Within Reference Range Is Inversely Associated With Alzheimer Disease

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Abbreviations: AD, Alzheimer disease; AF, atrial fibrillation; CHD, coronary heart disease; DTC, differentiated thyroid carcinoma; FT4, free thyroxine; GWAS, genome-wide association studies; IVW, inverse-variance weighted; MR, mendelian randomization; MR-PRESSO, mendelian randomization pleiotropy residual sum and outlier; OR, odds ratio; T4, thyroxine; TRH, TSH-releasing factor; TSH, thyrotropin.

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Abstract

Context: Contradictory findings were reported in observational studies on the association of thyroid function (thyrotropin [TSH] and free thyroxine [FT4] levels) with Alzheimer disease (AD).

Objective: This work aims to determine whether genetically determined TSH/FT4 levels within reference range are causally associated with AD.

Methods: A bidirectional, 2-sample mendelian randomization (MR) study was conducted. With summary statistics from the largest genome-wide association studies (GWAS)/GWAS meta-analysis of TSH level($n \geq 54\,288$), FT4 level($n = 49\,269$), and AD (71 880 cases; 383 378 controls), we used an MR approach to evaluate the bidirectional causal relationship between TSH/FT4 levels and AD. The inverse-variance weighted method was adopted as the main analysis.

Results: One SD increase in genetically determined TSH level within reference range was causally associated with a reduced risk of AD (odds ratio: 0.988; 95% CI, 0.977-0.998). A similar inverse association was observed in sex-specific analysis. The causal association was attenuated after adjustment for atrial fibrillation and blood pressure, suggesting they may mediate the causal pathway. A positive causal effect of AD on

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TSH level was detected only in male participants. This male-specific feedback loop may explain why the largest cohort study to date (Rotterdam Study) demonstrated a null observational association in men. Null association was observed between FT4 level and AD in both directions.

Conclusion: Genetic predisposition to increased TSH level, even within reference range, may lower the risk of AD, with atrial fibrillation, systolic, and diastolic blood pressure as possible mediators. Given the higher magnitude of risk reduction observed in the Rotterdam Study, whether the causal estimates derived from this MR study are underestimated warrants further investigation.

Key Words: thyrotropin, free thyroxine, Alzheimer disease, mendelian randomization

Alzheimer disease (AD) is the major cause of dementia but its pathogenesis remains largely unknown. Approximately 44.9 million people worldwide suffered from AD and other dementias in 2017 (1). Despite the rising burden, the licensed treatment of AD can just alleviate the symptoms (2). Thus, understanding the pathogenesis is urgently required for the development of disease-modifying treatments for AD.

Meanwhile, thyroid function is related to the neurodegenerative process, with cognitive impairment the leading cause of functional disability among older individuals and the major symptom of AD/other dementias (3). Contradictory findings have been reported on the association of thyroid function with risk of dementia/AD in several cross-sectional (4) or prospective studies of small sample size or short follow-up time (5-13). A null (6, 8, 10, 11), inverse (4, 7, 9, 12, 13), or U-shaped (5) association was observed between thyrotropin (TSH) level and risk of cognitive impairment/AD/dementia (5). Similar conflicting results were obtained for increased free thyroxine (FT4) level with risk of cognitive impairment/AD dementia (6, 8-13). The Rotterdam Study was the largest prospective cohort to date (13), demonstrating that higher TSH and FT4 levels, both in the full range and the reference range, were linked to reduced and elevated risk of incident dementia, respectively. Nevertheless, observational studies cannot infer causality.

Mendelian randomization (MR) is a powerful approach making use of genetic variation as a random experiment to evaluate the causal association of an exposure with an outcome, subject to fulfillment of several assumptions (Fig. 1) (14). In this study, causal effects of thyroid function (including TSH and FT4 levels within reference range) on AD, as well as the reverse causation, were evaluated using univariable MR analysis. Sex-specific analysis was performed to explore if there is any sex difference in the association. Multivariable MR was also conducted to examine the presence of potential mediators in the causal pathway.

Materials and Methods

Study Design and Data Sources

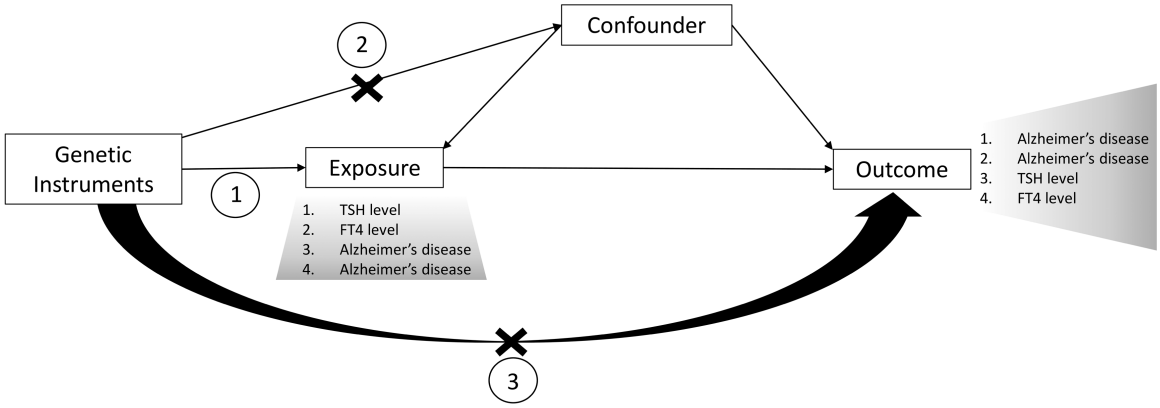
In MR studies, genetic variants that influence the susceptibility to an exposure could serve as the instruments for the exposure. Summary statistics of these instruments were extracted from the largest possible publicly available genome-wide association studies (GWAS)/GWAS meta-analysis of the exposure (forward causation: TSH and FT4 levels within reference range [15, 16]; reverse causation: AD [17]) and outcome (forward causation: AD; reverse causation: TSH and FT4 levels within reference range). As the Rotterdam and Framingham studies previously demonstrated a female-specific association of TSH level with incident dementia/AD (5, 13), genetic instruments from the sex-specific GWAS of TSH conducted by the ThyroidOmics Consortium (16) were also extracted to examine sex-specific effect. Thyroid function was previously reported to be associated with the risk factors of AD or dementia, including body mass index, type 2 diabetes, coronary heart disease (CHD), stroke, blood pressure, and atrial fibrillation (AF) (Supplementary Table 1) (18). It is unclear if these risk factors mediate the causal association, or thyroid function is an independent risk factor of AD. The aforementioned risk factors were included as potential mediators in multivariable MR analysis. The study design and details of the data sources are included in Fig. 1 and Supplementary Table 2 (18), respectively. The procedures in selecting the genetic instruments are detailed in Supplementary Methods 1(18), while the number of genetic instruments applied in each MR analysis are listed in Table 1.

Power Calculation

For continuous exposure, the proportion of variance explained by each genetic instrument was calculated by the formula: $2 \times (\text{minor allele frequency}) \times (1 - \text{minor allele frequency}) \times (\text{effect size})^2$, where effect size is in SD of

(a) Assumptions of univariable Mendelian Randomization

1. The genetic instruments are associated with the exposure.
2. The genetic instruments are not associated with any confounders that affects the exposure-outcome relationship.
3. The genetic instruments can only affect the outcome via the exposure. If the genetic instruments affect the outcome via other risk factors, it is known as horizontal pleiotropy.



(b) Assumptions of multivariable Mendelian Randomization

1. The genetic instruments are associated with the exposure or / and the mediators.
2. The genetic instruments are not associated with any confounders that affects the exposure-outcome relationship.
3. The genetic instruments can only affect the outcome via the exposure and / or mediators.

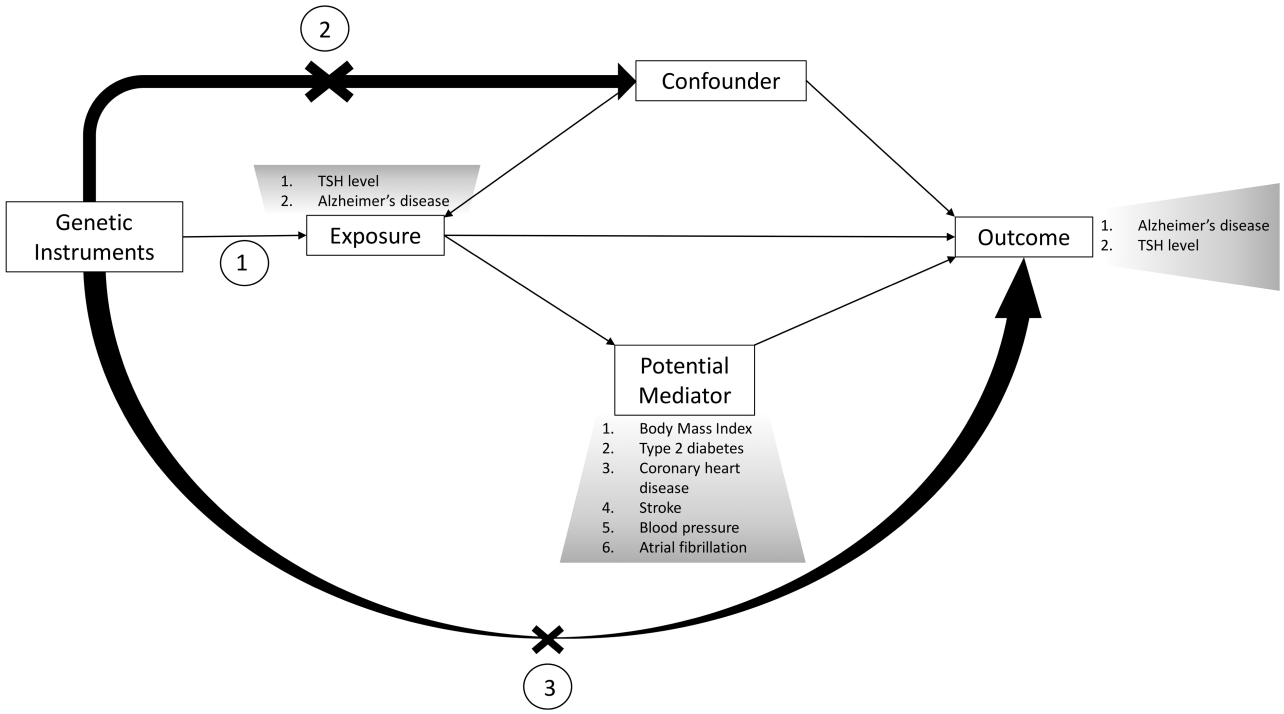


Figure 1. Key assumptions in mendelian randomization analyses and study design. A, Assumptions of univariable mendelian randomization. B, Assumptions of multivariable mendelian randomization.

exposure. For binary exposure, the proportion of variance explained by the genetic instruments was derived from the Mangrove package (19) in R, which also takes into account the disease prevalence. The *F* statistics, a measure of strength of genetic instruments for each MR analysis, are presented in Table 1. An online tool (https://sb452.shinyapps.io/power/) (20) was used to calculate power for each MR analysis. A plot of power against odds ratio (OR; for binary outcome of AD) or causal estimates (for continuous outcomes of TSH/FT4 levels) of the true

Table 1. Strength of genetic instruments of mendelian randomization analyses

	Exposure		Outcome		No. of genetic instruments in MR analysis(No. of independent genome-wide identified from GWAS– No. of instruments without proxies– No. of pleotropic instruments identified by PhenoScanner– No. of pleotropic outliers identified by MR-PRESSO)	Variance explained by instruments on exposure, %	*F* statistics
	Trait	No. of samples	Trait	No. of samples			
1	TSH (overall) (15)	119 715			82 (99 – 9 – 8 – 0)	10.18	13 569.23
2	TSH (overall) (16)	54 288			55 (61 – 2 – 4 – 0)	8.71	5180.63
3	TSH (women) (16)	24 790[a]		71 880 cases; 383 378 controls; Total: 455 258	34 (39 – 1 – 4 – 0)	7.10	1895.61
4	TSH (men) (16)	21 953[a]	AD (17)		38 (44 - 4 - 2 - 0)	8.83	2127.19
5	FT4 (overall) (16)	49 269			27 (31 – 0 – 2 – 2)	4.47	2306.37
6	FT4 (women) (16)	24 095[a]			15 (15 – 0 – 0 – 0)	3.39	846.48
7	FT4 (men) (16)	20 500[a]			11 (13 – 0 – 2 – 0)	2.71	572.02
8			TSH (overall) (15)	119 715	81 (94 – 5 – 8 – 0)	0.530	2426.78
9			TSH (overall) (16)	54 288	62 (94 – 25 – 7 – 0)	0.419	1917.35
10			TSH (women) (16)	24 790	62 (94 – 25 – 7 – 0)	0.413	1889.13
11	AD (17)		TSH (men) (16)	21 953	62 (94 – 25 – 7 – 0)	0.410	1873.96
12			FT4 (overall) (16)	49 269	64 (94 – 23 – 7 – 0)	0.427	1951.95
13			FT4 (women) (16)	24 095	61 (94 – 26 – 7 – 0)	0.406	1857.55
14			FT4 (men) (16)	20 500	64 (94 – 23 – 7 – 0)	0.427	1952.38

Abbreviations: AD, Alzheimer disease; FT4, free thyroxine; MR, mendelian randomization; MR-PRESSO, mendelian randomization pleiotropy residual sum and outlier; TSH, thyrotropin.
[a]The minimum sample size was used to calculate the strength of genetic instruments and power.

underlying association is shown in Supplementary Fig. 1 (18).

Mendelian Randomization Analyses

All the genetic instruments were oriented so that the effect alleles were positively associated with the exposure. The effect alleles were matched across the summary data of the exposure, potential mediator, and outcome data set. To facilitate matching, palindromic genetic instruments on ambiguous strands were replaced by nonpalindromic proxies in high linkage disequilibrium ($r^2 \geq 0.8$) (21) because of the unavailability of effect allele frequency of some GWAS data sets. A univariable inverse-variance weighted (IVW) method was used as the main MR analysis to evaluate the total effect of the exposure on the outcome (22, 23). A weighted median method (24) was employed as sensitivity analyses. The MR-Egger intercept test (25) and global test of MR-PRESSO (26) were used to detect for the presence of pleiotropy. In case univariable MR analysis suggested the presence of causal association, multivariable IVW analysis was also performed to dissect the mechanisms in the causal pathway from the exposure to the outcome (22, 27). Multivariable MR analysis was reported to estimate the direct causal effect of the exposure on the outcome by keeping the potential mediator constant. The presence of difference between the causal estimates of the univariable (total effect) and multivariable MR analysis (direct causal effect) implies that causal effect acts at least in part via the potential mediator (indirect effect) (22). A multivariable MR-Egger intercept test was applied to detect for presence of residual pleiotropy via other unmeasured risk factors (28). Different methods of MR analyses are elaborated in Supplementary Methods 2 (18).

In evaluating the reverse causation of AD on TSH and FT4 levels, the exposures were binary variables, and the causal estimates were initially equivalent to the change in the outcome per unit change in the exposure on the log odds scale (= exponential 1, ie, 2.72-fold change in the odds of the exposure). For better interpretation, the causal estimates were converted by multiplying 0.693 (= ln 2) to represent change in outcome per 2-fold change in the prevalence of the exposure (29).

Results

Two-Sample Mendelian Randomization of Thyrotropin Level and Alzheimer Disease

Using 82 genetic instruments for TSH level within reference range retrieved from the largest GWAS meta-analysis to-date (15), univariable MR analysis demonstrated that genetically increase in TSH level by 1 SD (1.036 mU/L)

was casually associated with lower risk of AD in the IVW method (OR = 0.988; 95% CI, 0.977-0.998; P = .017), and the sensitivity analysis of weighted median method (OR = 0.982; 95% CI, 0.968-0.997; P = .021; Fig. 2A). Similar results (IVW analysis: OR = 0.986; 95% CI, 0.976-0.996; $P = 8.11 \times 10^{-3}$; weighted median method: OR = 0.982; 95% CI, 0.968-0.997; P = .017; see Fig. 2A) were obtained using 55 genetic instruments derived from the GWAS meta-analysis conducted by the ThyroidOmics Consortium (16), which was a subset of the aforementioned largest GWAS meta-analysis (15). Despite its smaller sample size, sex-specific summary statistics was available for this data set. Genetic predisposition to increased TSH level both in women and men was associated with reduced risk of AD (IVW analysis in women: OR = 0.985; 95% CI, 0.975-0.996; $P = 8.39 \times 10^{-3}$; IVW analysis in men: OR = 0.985; 95% CI, 0.976-0.995; $P = 2.52 \times 10^{-3}$; see Fig. 2A). A similar OR was obtained using the weighted median method (see Fig. 2A).

Using the multivariable MR approach, we examined if the causal association of genetically determined TSH level with AD was mediated by potential mediators. This analysis was performed using genetic instruments representing TSH level within reference range in the whole study population (15, 16), women (16), and men (16), respectively. In all these analyses, the causal association of genetically determined TSH level with AD was attenuated after adjustment for AF (Fig. 2B). In the male-specific analysis, the association was also attenuated after adjustment for systolic and diastolic blood pressure, but not other potential mediators (see Fig. 2B).

For the reverse direction, univariable IVW analysis demonstrated a null association of AD with TSH level in the whole study population and in women (Fig. 2C). In male-specific analysis, per genetically doubling the odds of AD, a positive causal association with TSH level was detected (IVW:

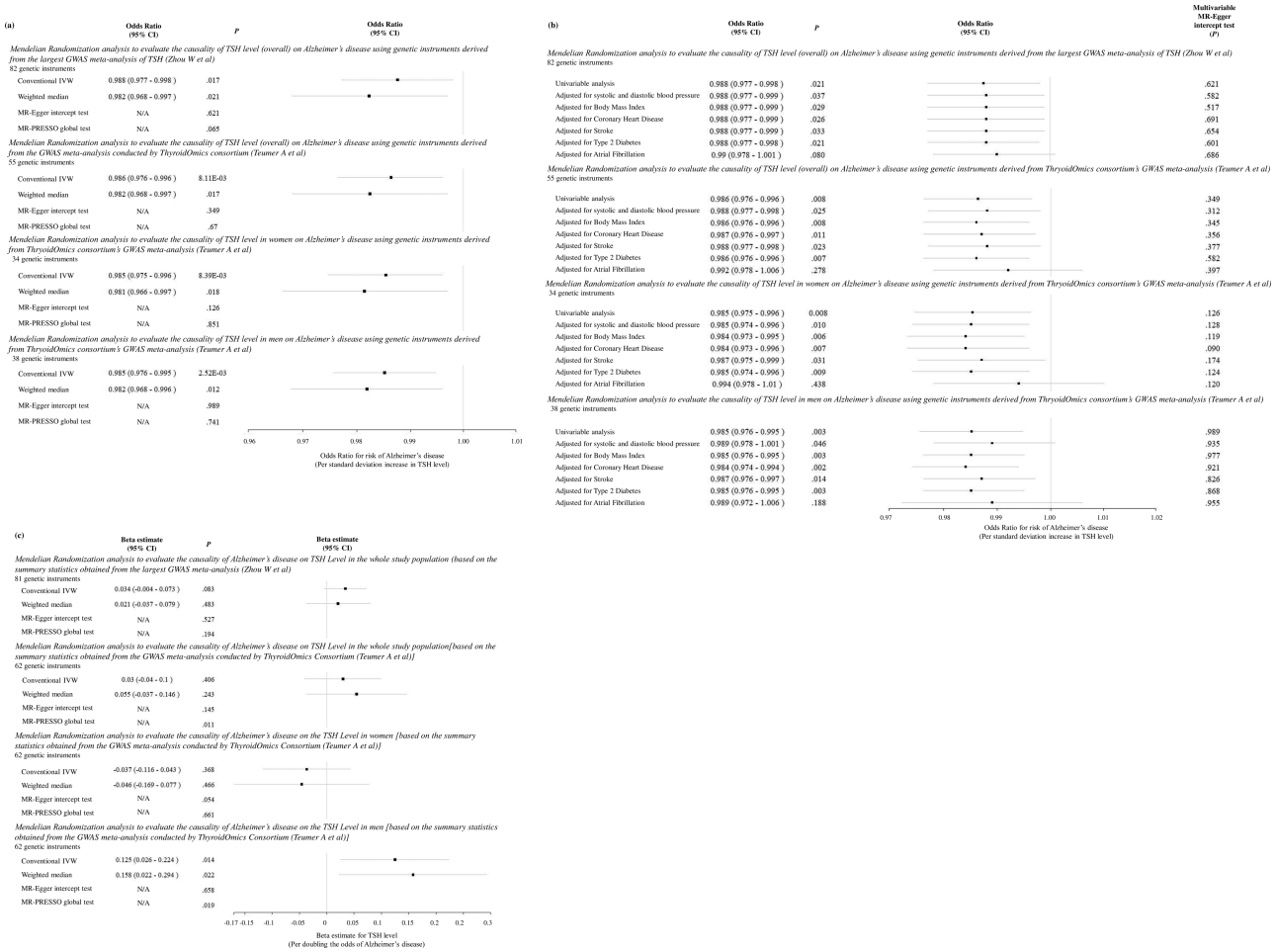


Figure 2. Result of mendelian randomization (MR) analysis in evaluating the causal association between thyrotropin (TSH) level and Alzheimer disease. A, Odds ratio of Alzheimer disease per SD increase in TSH level in univariable MR analysis. B, Odds ratio of Alzheimer disease per SD increase in TSH level in multivariable MR analysis. C, Causal estimate for TSH level per doubling the odds of Alzheimer disease in univariable MR analysis.

β = 0.125; 95% CI, 0.026-0.224; *P* = .014; weighted median: β = 0.158; 95% CI, 0.022-0.294; *P* = .022; see Fig. 2C).

In all the univariable and multivariable analyses, the MR-Egger intercept tests were insignificant (*P* > .05; see Fig. 2). The MR-PRESSO global tests were all insignificant (*P* > .05), except in evaluating the causality of genetic predisposition to AD on TSH level in the whole study population and men by using the ThyroidOmics Consortium's summary statistics (see Fig. 2C), but no significant outliers were detected.

Two-sample Mendelian Randomization of Free Thyroxine Level and Alzheimer Disease

With 27, 15, and 11 instruments representing genetically determined FT4 level in the whole study population, women, and men, respectively, univariable MR analyses demonstrated FT4 level had a null association with AD (Fig. 3A). For the reverse direction, AD also had a null association with FT4 level (Fig. 3B). MR-Egger intercept tests were all insignificant. While the MR-PRESSO global test was significant in the MR analysis investigating the causal association of AD with FT4 level in women, no significant outliers were identified. The MR-PRESSO global tests were insignificant in all other analyses (see Fig. 3).

Discussion

In this 2-sample, univariable MR study, we demonstrated an inverse causal association of genetically determined circulating TSH level within reference range with risk of AD, implying that genetically increased and decreased TSH level within reference range might reduce and increase risk of AD, respectively. Using a multivariable MR approach, we revealed that AF, systolic, and diastolic blood pressure might mediate the causal pathway from TSH to AD. While a null association was observed in the reverse direction between AD and TSH level in the whole study population and the female subgroup, a positive causal association existed in male participants. Insufficient evidence was available to support any causal relationship between genetically determined circulating FT4 level and AD in both directions.

This is the first MR study that evaluated the causal relationship of thyroid function with risk of AD. We first inferred causality between genetically determined circulating TSH level within reference range and AD (per genetic increase in TSH level by 1 SD, OR for risk of AD = 0.988) using the genetic instruments retrieved from the largest GWAS meta-analysis of TSH level to date (15). The inverse association was also detected in the whole study population, female, and male participants in additional sensitivity analysis and sex-specific analysis employing genetic instruments from the ThyroidOmics Consortium's GWAS meta-analysis of TSH (16) (a subset of the largest GWAS meta-analysis). In all these analyses, similar ORs for risk of AD were obtained from the primary conventional IVW method, as well as the sensitivity analysis of the weighted median method, providing robust evidence of the causal link between genetically increased TSH level and reduced risk of AD. Such an inverse causal association is in line with the Rotterdam Study that higher TSH level in the reference range of thyroid function was associated with a reduced risk of dementia, whereas no difference in risk estimates for AD and other dementia was observed in the stratified analysis (13). In comparison with the causal estimates derived from our MR study, the Rotterdam Study had a greater magnitude of hazard ratio (per unit increase in log[TSH], hazard ratio = 0.76; 95% CI, 0.64-0.91). The possible underestimation of effect size in MR analyses was also observed in other studies. For instance, an MR study found that the lifelong exposure to 1 mmol/L lower low-density lipoprotein cholesterol by the genetic proxy of statins near its target gene, 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*), was associated with a reduced risk of CHD by 6% (30). Meanwhile, a meta-analysis of statin trials demonstrated that the same reduction of low-density lipoprotein cholesterol was associated with a 24% reduction of CHD risk (30). Whether the causal estimate derived by the present MR study is underestimated warrants further investigation. Moreover, the association of TSH level with AD was observed only in women in the Rotterdam Study (13). Such a discrepancy may also be due to the limitation of observational studies that the findings are subjected to reverse causality, which can be overcome by an MR approach. In our univariable MR analysis in the reverse direction, we found positive causal effects of AD on TSH level in men only, indicating the potential presence of a male-specific feedback loop (illustrated in Supplementary Fig. 2) (18). We hypothesize that in men, genetically increased TSH level within the reference range might causally reduce the risk of AD, which might subsequently lower the TSH level, leading to insubstantial changes in the overall TSH level and hence AD risk. This may explain the null observational association between TSH level and AD in men in the Rotterdam Study. Further investigation is warranted to validate this hypothesis.

A number of mechanisms have been proposed for the association of high thyroid function with AD. One proposed mechanism is the direct effects of thyroid hormones on the expression of amyloid β proteins, phosphorylation of tau proteins, increased oxidative stress, and neuronal death (31), but these cannot be proved by an MR approach because of the unavailability of genetic data. Another possible mechanism is through vascular risk factors (31),

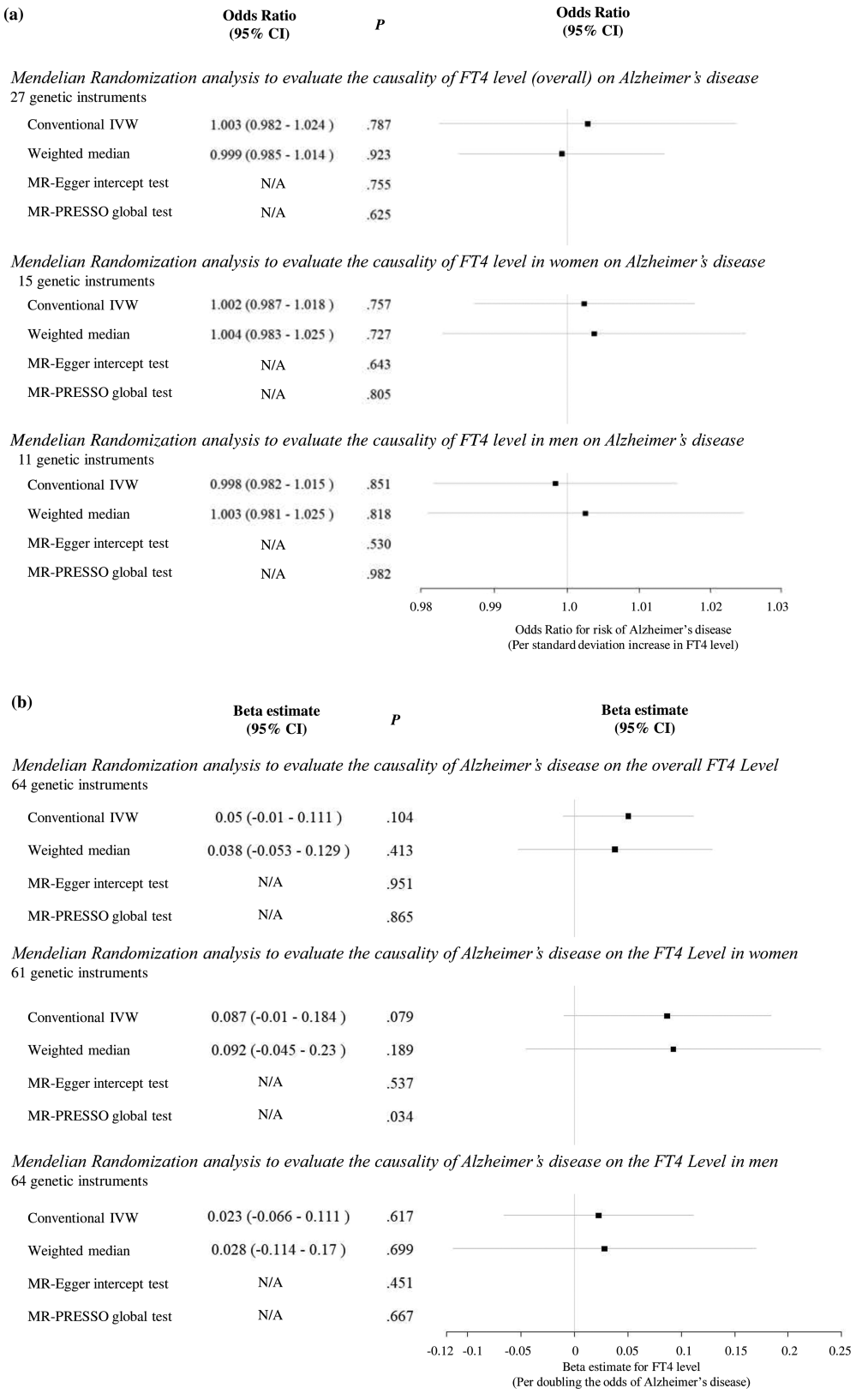


Figure 3. Result of mendelian randomization (MR) analysis in evaluating the causal association between free thyroxine (FT4) level and Alzheimer disease. A, Odds ratio of Alzheimer disease per SD increase in FT4 level in univariable MR analysis. B, Causal estimate for FT4 level per doubling the odds of Alzheimer disease in univariable MR analysis.

which may subsequently lead to vascular brain damage. Using a multivariable MR approach, we examined if some of these vascular risk factors (including systolic and diastolic blood pressure, body mass index, CHD, stroke, type 2 diabetes, and AF) may mediate the causal pathway from TSH level to AD. In the whole study population and sex-specific analyses using different sets of genetic instruments, the inverse causal effect of genetically determined TSH level on AD was attenuated after adjustment for AF, suggesting AF might partly mediate the causality. Patients with hyperthyroidism (characterized by a low TSH level) had increases in septal and posterior wall, left ventricular mass (32) and contractility (33), elevated rate of blood flow across the aortic valve (33), and impaired left ventricular relaxation (34), which all contribute to the development of AF. Meanwhile, patients with AF had reduced cerebral blood flow velocity of the middle cerebral artery and decreased cerebral perfusion (35), which were associated with accelerated cognitive decline and increased risk of dementia (36). While an MR study revealed that genetically decreased TSH level within the reference range was associated with an elevated risk of AF (37), another study did not find evidence to support the causal association of genetically predicted AF with AD (38). We hypothesize that genetic predisposition to AF specifically induced by genetically decreased TSH may be causally associated with AD development. Further research is required to validate this hypothesis, if access to the thyroid function of individuals included in the GWAS of AF becomes available. On the other hand, systolic and diastolic blood pressure attenuated the causality of genetically determined TSH level and AD in the male-specific analysis, implying its potential mediating role. By dilating the resistance arterioles, reducing systemic vascular resistance, increasing heart rate, and raising cardiac output, hyperthyroidism is a secondary cause of hypertension (39). Hypertension directly causes vessel wall changes, hypoperfusion, and hypoxia of the brain, which are reported to induce neurodegeneration of AD (40). Hypertension also increases the risk of AD indirectly via atherosclerosis and impairing blood flow (39). Nevertheless, the potential aforementioned mechanisms were derived from studies involving individuals with overt hyperthyroidism. While contradictory findings were reported by observational studies on the mediating role of vascular pathways in the association of TSH level with dementia/AD (5, 13, 41), the detailed mechanisms of how variation of TSH level within the reference range may inversely and causally affect the risk of AD will require future investigation. On the other hand, little effort has been made to investigate the reverse causation on how AD might alter TSH level. One proposed mechanism is that involutional changes of the brain observed in people with AD might cause the perturbation of neurotransmitters, including TSH-releasing factor (TRH) (42). The reduced secretion of TRH from the hypothalamus might subsequently lead to lower TSH level. Nevertheless, limited evidence was available to support or invalidate this mechanism (42), and its direction was the opposite of the positive male-specific causal association of AD with TSH level as uncovered in this study. To our knowledge, no GWAS of TRH level has been published to date so we cannot verify this mechanism by multivariable MR approach. Further examination will be needed to elucidate the underlying mechanisms.

The present study findings may be clinically important. The inverse causality between genetically reduced TSH level within reference range and increased AD risk implies that clinicians may need to pay attention to the medication compliance of patients with overt hyperthyroidism, especially those with mild symptoms, because noncompliance may drive their TSH level toward the lower end of the reference range, thus increasing the risk of AD. Our study finding also brings up the question of whether thyroxine treatment for patients with hypothyroidism would increase the risk of AD, as some practitioners intend to set the treatment target of TSH level to the lower end of the reference range because of the skewed distribution of TSH level in the healthy population (43). Yet, the clinical guidelines do not recommend targeting the TSH level toward the lower end of the reference range (43), since a randomized controlled trial demonstrated that the cognitive outcomes of patients with TSH level at the lower end of the reference range were not better than others with TSH level toward the upper end (44). Owing to the possible elevated risk of AD with lower TSH level, our study findings support the guideline recommendations. Nonetheless, the causal association implied in this MR analysis refers to the endogenous lifelong TSH level that is genetically determined. It remains unclear if altered TSH level resulting from thyroxine treatment to the normal to low level would affect the risk of AD. It is also unknown if change in the TSH level at different stages of the life course would influence the subsequent risk of AD. While a previous longitudinal study with 499 relatively older volunteers (mean age, 76.9 years) showed that individuals using thyroid hormone replacement therapy progressed to diagnosis of AD more rapidly than those not taking the therapy (45), a cross-sectional study of small sample size showed that differentiated thyroid carcinoma (DTC) patients aged 65 years and older who received TSH-suppressive therapy for at least 5 years had comparable cognitive function with matched controls without DTC (46). Yet, the mean TSH levels of the DTC patients in the latter study were below their study-specific reference range, although their FT4 level was within the range and positively correlated with a few cognitive functions tested

among the DTC patients (46). Whether regulation of TSH level by thyroxine treatment to normal to low levels will influence risk of AD requires large-scale, population-based pharmacoepidemiology studies.

The major strength of this study is that the MR approach is less subjected to reverse causality and residual confounding when compared to conventional observational studies, enabling the evaluation of causal inference between the exposure and the outcome. In particular, the use of 2-sample MR analysis (with data of exposure and outcome taken from 2 data sets) was reported to provide a less biased causal estimate (47). Even if bias is present, it is in the direction of the null (47). Thus, the inverse causal association of genetically determined TSH level within the reference range with risk of AD supported by multiple sensitivity analyses in this MR study is likely to be genuine. As the summary statistics of the genetic instruments were retrieved from the largest possible GWAS/GWAS meta-analysis of the exposure and outcome, our MR analysis is well powered (Supplementary Fig. 1) (18). The *F* statistics of the genetic instruments were relatively high (see Table 1), indicating that weak instrument bias is unlikely.

There are also limitations to the present study. First, the effect estimate of the detected causal association between genetically determined TSH level and AD was relatively small. Nevertheless, it was suggested that the assumption of linearity of the exposure-outcome association should be complied for accurately estimating the magnitude of the causal effect, although this assumption would not affect assessing the presence of a causal relationship (48). As the U-shaped relationship between TSH and AD was suggested in the Framingham Study (5), the magnitude of causal effect estimated in this MR study may require cautious interpretation, despite the confirmed presence of a causal relationship. In addition, because of the intrinsic difference between genetic variants and pharmacological interventions on the exposure (such as mechanism, magnitude, and duration of intervention), it was recommended that the causal effect estimate obtained from MR studies should not be directly interpreted as the outcome of an intervention on the exposure (48). Secondly, owing to the unavailability of individual-level genetic data, we cannot investigate the U-shaped relationship of TSH level and AD as suggested in the Framingham Study (5). If the genuine causal relationship is U shaped, the magnitude of the causal effect in our study is likely underestimated. Third, it is possible that the genetic instruments act on the outcome via some unknown pathways other than the exposure, violating one of the MR assumptions. To address this issue, we employed MR-Egger intercept and MR-PRESSO global tests to detect horizontal pleiotropy. Both tests were insignificant in the MR analysis evaluating the causality between genetically determined TSH level and AD, suggesting the observed inverse causality is unlikely due to pleiotropy, though it cannot be completely ruled out. In evaluating the causal association of AD with TSH (in whole study population and male subgroup) and FT4 (in female subgroup), the MR-PRESSO global test was significant. However, pleiotropic outliers were not identified, and a MR-PRESSO distortion test could not be conducted. In addition to the insignificant MR-Egger intercept test, we consider the results were not indicative of horizontal pleiotropy, although we cannot preclude the possibility that the observed causal association may be due to pleiotropy. Fourth, in the MR analysis stratified by sex, we used the summary statistics obtained from the sex-specific GWAS meta-analysis of TSH level within reference range (16), but sex-specific GWAS analysis of AD is unavailable to date. The sex-specific MR analysis should be revisited when such genetic data become available. Fifth, GWAS of 3,5,3′-triiodothyronine and autoimmune antibodies, such as thyroid peroxidase antibodies, are unavailable. Because 3,5,3′-triiodothyronine and thyroid peroxidase antibodies also play a role in thyroid function, future MR studies examining their causal relationship with AD are warranted. Last, we attempted to identify the potential mediators in the causal pathway from TSH level to AD by multivariable MR analysis. Yet, the list of potential mediators under investigation is not exhaustive. Further studies on additional potential mediators are required.

This study provides evidence that genetically determined TSH level, even within the reference range, is inversely and causally associated with risk of AD. Such findings may have clinical implications. First, clinicians may need to pay attention to the medication compliance of patients with overt hypothyroidism. Otherwise, patient TSH levels may fall toward the lower end of the reference range, thus increasing the risk of AD. Second, the findings suggest that the low TSH level in response to thyroxine treatment in patients with hypothyroidism may elevate the risk of AD. Further investigations are required to confirm the hypothesis.

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Author Contributions: G.H.L. and C.L.C. conceived the study. G.H.L. conducted the statistical analysis and drafted the manuscript. All coauthors were involved in interpreting the data and revising the manuscript for final submission.

Additional Information

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Disclosures: The authors have nothing to disclose.

Data Availability: The present MR study uses publicly available summary statistics from GWAS/GWAS meta-analysis that can be downloaded from the websites of the corresponding consortiums. The data sources are detailed in Supplementary Table 2 (18).

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