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Abstract

Objective: We aimed to validate the association of genome-wide association study (GWAS)-identified loci and polygenic risk score with serum thyroid-stimulating hormone (TSH) concentrations and the diagnosis of hypothyroidism. Then, the causal relationship between serum TSH and osteoporotic bone fracture risk was tested. ***Methods:*** A cross-sectional study was done among patients of European Caucasian ethnicity recruited in Tayside (Scotland, UK). Electronic medical records (EMRs) were used to identify patients and average serum TSH concentration and linked to genetic biobank data. Genetic associations were performed by linear and logistic regression models. One-sample Mendelian randomization (MR) was used to test causality of serum TSH on bone fracture risk. ***Results:*** Replication in 9,452 euthyroid individuals confirmed known loci previously reported. The 58 polymorphisms accounted for 11.08% of the TSH variation ($p <$ 1e−04). TSH-GRS was directly associated with the risk of hypothyroidism with an odds ratio (OR) of 1.98 for the highest quartile compared to the first quartile ($p = 2.2$e−12). MR analysis of 5,599 individuals showed that compared with those in the lowest tertile of the TSH-GRS, men in the highest tertile had a decreased risk of osteoporotic bone fracture (OR = 0.59, $p = 2.4$e−03), while no difference in a similar comparison was observed in women (OR = 0.93, $p = 0.61$). Sensitivity analysis yielded similar results. ***Conclusions:*** EMRs linked to genomic data in large populations allow replication of GWAS discoveries without additional genotyping costs. This study suggests that genetically raised serum TSH concentrations are causally associated with decreased bone fracture risk in men.

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Introduction

Genome-wide association studies (GWASs) have found association signals with serum thyroid-stimulating hormone (TSH) concentrations and/or hypothyroidism [1–4]. Replication studies in independent samples are scarce but desirable to help ensure that such signals are relevant [5].

Replication of an association requires genotyping the initially discovered genetic variant in a completely inde-

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pendent sample of sufficient size. Recently, electronic medical record (EMR)-derived phenotypes are being linked to genetic biobanks to allow research on the genetic basis of a wide range of traits highly cost-effectively. Therefore, such EMR-linked biobanks might be appropriate to investigate the role that genetics play in thyroid-related disorders.

A recent meta-analysis of GWAS for thyroid function and dysfunction, testing up to 8 million genetic variants, developed a TSH-based genetic risk score (GRS) and reported its association with hypothyroidism, hyperthyroidism, and other endpoints which may be associated with thyroid disease [3]. Whether this TSH-GRS may be useful to investigate causal relationships between modifiable risk factors and disease outcomes also needs to be supported by further confirmatory studies [6].

We aimed to validate the association of GWAS-identified loci and polygenic risk score with serum TSH concentrations and the diagnosis of hypothyroidism in a Scottish population. Mendelian randomization (MR) was used to test the hypothesis that serum TSH concentrations causally influence the risk of osteoporotic bone fractures, as previous studies have shown an association [7].

Methods

A cross-sectional study was done among individuals from the Genetics of Diabetes and Audit Research Tayside Study (GoDARTS) recruited in Tayside, Scotland (UK). All subjects in this population are of white ethnicity and have previously been described [8]. EMRs (biochemistry, prescribing, hospital admissions, and demographics) were used to ascertain euthyroid and hypothyroid patients, identify those with osteoporotic bone fractures, and were anonymously linked to genetic biobank data by the Health Informatics Centre of the University of Dundee (http://www.dundee.ac.uk/hic). Patients issued with at least 2 prescriptions for L-thyroxine (British National Formulary codes-BNF 6.2.1) during the study period (1994–2014) were defined as being hypothyroid. Patients with previous hyperthyroidism, identified from having previous thyroid surgery history by OPCS Classification of Surgical Operations and Procedures (OPCS4: B08, B09, B12), radioactive iodine, and/or a prescription of anti-thyroid drug use (BNF 6.2.2), or who had thyroid cancer from ICD codes (ICD9: 193; ICD10: C73, D093, D440) were excluded. The median serum TSH recorded throughout the study period for each patient was used. Euthyroid subjects had an average serum TSH concentration of 0.4–4.0 mIU/L. The ICD codes for fractures of the vertebral column, forearm, and hip (ICD9: 733.1, 805.4, 805.5, 806.4, 806.5, 813, 820–21; ICD10: M80, S32, S52, and S72) were considered as osteoporotic fractures [9].

Imputation and imputation quality of data genotyped by different platforms were previously described [10]. In brief, genotype data were available from the following platforms: the Hu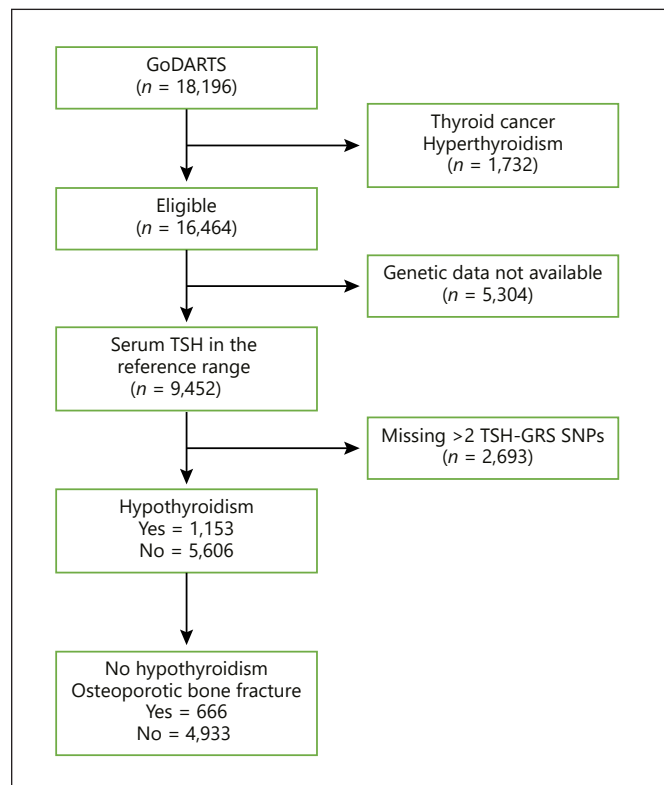man Exome-12 VI_A_chip, the Metabohip, Illumina HumanOmni Express-12 VI platform (Illumina, San Diego, CA, USA), Affymetrix 6.0 platform (Affymetrix, Santa Clara, CA, USA), and the Illumina Infinium custom GWAS chip (Illumina, San Diego, CA, USA). Imputation was performed against 1000G Phase I V3 reference panel using Impute2 and using the haplotype reference consortium [11]; calls made with imputation quality below 90% were discarded. All single-nucleotide polymorphisms (SNPs) were in Hardy-Weinberg equilibrium ($p < 10e{-}04$).

Genetic tests of association were performed by linear and logistic regression models. Linear regression models were used on euthyroid subjects to test the association with serum TSH concentration and to estimate the variation of TSH explained by the SNPs. To assess the consistency of effects of this study with those previously reported, meta-analyses were performed, and heterogeneity was quantified using the I^2 measure [12]. The combined effects of the genotypes were researched by GRS analyses using a weighted sum of TSH-increasing alleles across the 58 SNPs reported by Teumer et al. [3] available in our cohort and using weights from an external source. Participants missing >2 of these SNPs were excluded from the analyses. Association with hypothyroidism was performed on cases and controls by logistic regression. Odds ratios (ORs) from logistic models were adjusted for age at first TSH recording and gender.

Fig. 1. Flowchart describing the study cohort's generation process and the patients included in this study. SNP, single-nucleotide polymorphism; TSH, thyroid-stimulating hormone; TSH-GRS, TSH-based genetic risk score; GoDARTS, Genetics of Diabetes and Audit Research Tayside Study.

Fig. 2. Associations of TSH-based GRS with the probability of hypothyroidism (**a**) and osteoporotic-related bone fractures (**b**). Histogram represents the distribution of the polygenic risk score in the study sample. TSH, thyroid-stimulating hormone; GRS, genetic risk score; GoDARTS, Genetics of Diabetes and Audit Research Tayside Study; SNP, single-nucleotide polymorphism.
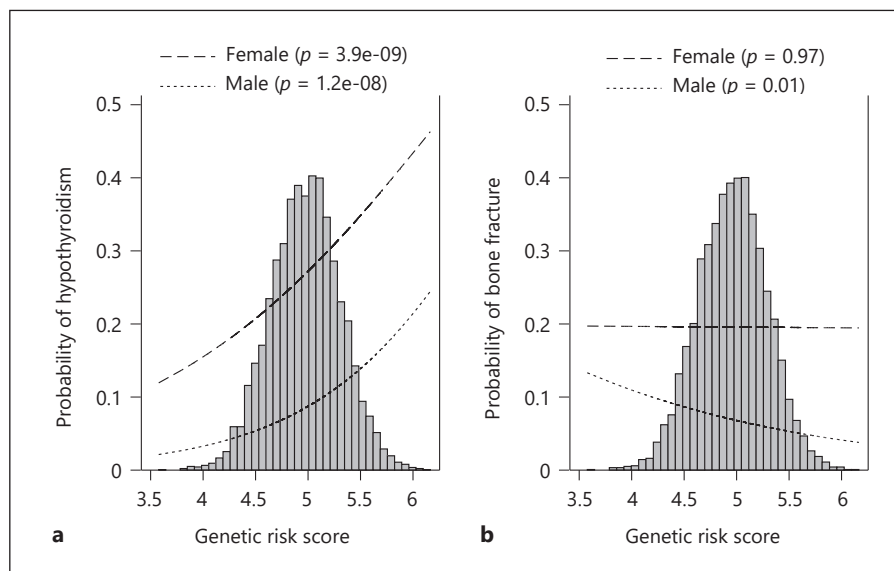
To test the hypothesis that circulating TSH concentrations causally influence the risk of osteoporotic bone fracture, we used a one-sample MR design with the instrumental variable approach using the 2-stage method [13]. It comprised 2 regression stages: the first-stage linear regression of the serum TSH on the instrumental variable (i.e., GRS) and the second-stage regression of the osteoporotic bone fracture on the predicted values of the serum TSH from the first stage (i.e., unconfounded estimate of TSH concentration attributed to these genotypes), where the second stage used an age-adjusted logistic regression model with robust variance to estimate a causal OR parameter. Sensitivity analysis was performed by excluding potential pleiotropic SNPs from the GRS in our MR analyses. The associations of the genetic variants with potential confounders (bone mineral density [BMD]) were annotated using the PhenoScanner database with the default search options [14, 15]. All statistical analyses were conducted using STATA/SE version 13.1 software (StataCorp, College Station, TX, USA).

Results

We identified 16,464 individuals as being eligible for the study after exclusion of thyroid cancer and hyperthyroidism cases, of which 9,452 had serum TSH within the reference range and had available genomic data in the GoDARTS biobank (see Fig. 1). Hypothyroid cases were more likely to be female (73 vs. 43.4%, $p < 1e-03$) and had a higher average serum TSH concentration (2.2 vs. 1.7 mIU/L, $p < 1e-03$) than nonhypothyroid controls, but there was no difference in age (57 years).

For all participants with a serum TSH in the reference range ($n = 9,452$), we confirmed the association of serum TSH with known loci previously reported at *CAPZB*,

NFIA, *VAV3*, *IGFBP5*, *SYN2*, *NR3C2*, *PDE8B*, *VEGFA/LOC100132354*, *PDE10A*, *NRG1*, *GLIS3*, *PRDM11*, *ITPK1*, *FAM227B/FGF7*, *DET1*, *MAF*, *INSR*, and *FOXA2* (Table 1). Each copy of the TSH-increasing allele of rs2127387 at *PDE8B* (phosphodiesterase type 8B) was associated with an increase of 0.13 mIU/L serum TSH. This SNP accounted for 1.64% of serum TSH variation, followed by signals in the phosphodiesterase type 10A (*PDE10A*-rs1079418) and the capping protein-actin filament muscle Z-line β (*CAPZB*-rs10917469) that contributed to 0.70% and 0.62% of variation, respectively. Further adjustment of regression models for age and gender did not change the size and direction of the effect estimates. We also validated novel loci at *DIRC3*, *IGF2BP2*, *PSORS1C1*, *SLC25A37*, *SULF1*, *TG*, *C9orf92*, *GATA3*, *SPATA13*, *TSHR*, *MIR365A*, and *BCAS3*. When combined, the 58 SNPs accounted for 11.08% ($n = 2,089$, $p < 1e-04$) of the variation in serum TSH concentration that increased to 11.65% after also including age and gender as predictors in the linear model, thus leaving 0.57% (i.e., 11.65–11.08%) of the variation to age and gender. Male gender was associated with a lower serum TSH (β = −0.043, $p = 1.8e-02$), and each additional year of life conferred an increase of 0.005 mIU/L serum TSH ($p = 3.5e-05$). Although an I^2 value of 0% (i.e., no observed heterogeneity) was obtained in the majority of the meta-analyses, a significant heterogeneity was detected in 5 (Table 1; *SASH1*-rs9497965, *ABO*-rs8176645, *MBIP*-rs398745, *SOX9*-rs1042673, and *HES1*-rs59381142 showed an effect about 4 times smaller in our study). Euthyroid individuals carrying greater numbers of serum

Table 1. Association of SNPs with average TSH levels in euthyroid subjects

SNP	Chr	Gene	Coded allele	GoDARTS				Prior association[1]				Meta-analysis		
				freq	N	β (SE)†	% var	freq	N	β (SE)†	% var	β (SE)	Het. p value	I^2, %
rs12089835	1	CAPZB	T	0.38	6,817	0.055 (0.013)	0.26	0.35	51,018	0.073 (0.007)	0.23	0.069 (0.006)	0.223	32.70
rs10917469	1	CAPZB	A	0.84	6,836	0.114 (0.017)	0.62	0.84	51,018	0.111 (0.009)	0.32	0.112 (0.008)	0.876	0.00
rs74804879	1	CAPZB	T	0.68	5,660	0.022 (0.014)	0.04	0.65	51,018	0.050 (0.007)	0.11	0.044 (0.007)	0.074	68.80
rs334725	1	NFIA	A	0.95	6,818	0.150 (0.030)	0.36	0.95	51,018	0.174 (0.015)	0.27	0.169 (0.013)	0.474	0.00
rs17020122	1	VAV3	T	0.08	6784	0.074 (0.023)	0.15	0.09	51,018	0.104 (0.011)	0.16	0.098 (0.010)	0.239	27.80
rs16856540	2	IGFBP5	T	0.84	6,763	−0.022 (0.017)	0.02	0.84	52,047	−0.055 (0.008)	0.08	−0.049 (0.007)	0.079	67.60
rs13015993	2	IGFBP5	A	0.72	6,820	0.074 (0.014)	0.39	0.73	52,047	0.082 (0.007)	0.26	0.080 (0.007)	0.609	0.00
rs1663070	3	SYN2	T	0.75	6,780	−0.055 (0.014)	0.2	0.74	54,288	−0.046 (0.007)	0.08	−0.048 (0.006)	0.565	0.00
rs6535624	4	NR3C2	A	0.43	6,797	0.042 (0.013)	0.16	0.44	52,047	0.042 (0.006)	0.08	0.042 (0.006)	1.000	0.00
rs11732089	4	NR3C2	T	0.79	6,829	0.091 (0.015)	0.5	0.80	52,047	0.115 (0.008)	0.42	0.110 (0.007)	0.158	49.80
rs62362610	5	PDE8B	C	0.08	6,552	0.096 (0.023)	0.26	0.08	53,074	0.073 (0.012)	0.08	0.078 (0.011)	0.375	0.00
rs1119208	5	PDE8B	T	0.34	9,383	0.039 (0.011)	0.13	0.37	53,074	0.046 (0.006)	0.09	0.044 (0.006)	0.576	0.00
rs139424329	5	PDE8B	A	0.01	6,794	−0.072 (0.060)	0.02	0.01	45,981	−0.200 (0.032)	0.11	−0.172 (0.029)	0.060	71.80
rs2127387	5	PDE8B	A	0.38	6,805	0.138 (0.012)	1.64	0.41	53,074	0.144 (0.006)	0.99	0.143 (0.005)	0.655	0.00
rs7702192	5	PDE8B	A	0.44	6,806	0.082 (0.012)	0.61	0.47	53,074	0.070 (0.006)	0.24	0.072 (0.006)	0.371	0.00
rs113974964	5	PDE8B	T	0.05	6,824	−0.105 (0.029)	0.19	0.05	53,074	−0.124 (0.015)	0.13	−0.120 (0.013)	0.561	0.00
rs139149784	5	PDE8B	A	0.01	6,626	0.093 (0.048)	0.05	0.03	49,018	0.156 (0.029)	0.12	0.139 (0.025)	0.261	20.80
rs182873197	5	PDE8B	T	0.04	5,705	−0.047 (0.034)	0.03	0.05	53,074	−0.080 (0.014)	0.06	−0.075 (0.013)	0.369	0.00
rs744103	6	VEGFA/LOC100132354	A	0.66	6,786	0.075 (0.013)	0.45	0.69	54,288	0.092 (0.007)	0.36	0.088 (0.006)	0.250	24.60
rs9381266	6	VEGFA/LOC100132354	T	0.75	6,722	0.045 (0.015)	0.13	0.74	54,288	0.073 (0.007)	0.20	0.068 (0.006)	0.091	65.10
rs9497965	6	SASH1	T	0.4	6,646	0.015 (0.013)	0.02	0.40	54,288	0.044 (0.006)	0.09	0.039 (0.006)	0.043	75.60
rs73022105	6	PDE10A	T	0.95	6,741	0.068 (0.031)	0.07	0.95	52,424	0.105 (0.016)	0.09	0.097 (0.014)	0.289	11.10
rs1079418	6	PDE10A	A	0.7	6,789	0.096 (0.013)	0.7	0.69	52,424	0.101 (0.007)	0.43	0.100 (0.006)	0.735	0.00
rs2439301	8	NRG1	A	0.22	6,303	−0.038 (0.016)	0.09	0.23	54,288	−0.059 (0.008)	0.12	−0.055 (0.007)	0.240	27.40
rs10814915	9	GLIS3	T	0.41	9,452	0.046 (0.011)	0.19	0.44	54,288	0.042 (0.006)	0.08	0.043 (0.005)	0.750	0.00
rs8176645	9	ABO	A	0.26	4,795	0.013 (0.017)	0.01	0.34	54,288	0.052 (0.006)	0.12	0.048 (0.006)	0.031	78.60
rs200574439	10	NKX2-3	A	–			–	0.58	54,288	−0.047 (0.006)	0.10	−0.047 (0.006)	–	0.00
rs12284404	11	PRDM11	A	0.29	6,821	−0.058 (0.013)	0.26	0.27	54,288	−0.067 (0.007)	0.17	−0.065 (0.006)	0.542	0.00
rs398745	14	MBIP	A	0.57	6,813	−0.013 (0.013)	0.02	0.59	54,288	−0.052 (0.006)	0.13	−0.045 (0.006)	0.006	86.50
rs2254613	14	MBIP	T	0.51	6,541	−0.019 (0.013)	0.03	0.55	54,288	−0.035 (0.006)	0.05	−0.032 (0.005)	0.264	19.90
rs8015085	14	ITPK1	A	0.19	6,804	0.060 (0.016)	0.2	0.21	54,288	0.067 (0.008)	0.15	0.066 (0.007)	0.696	0.00
rs17477923	15	FAM227B/FGF7	T	0.73	6,798	0.075 (0.014)	0.4	0.74	54,288	0.083 (0.007)	0.26	0.081 (0.007)	0.609	0.00
rs11639111	15	FAM227B/FGF7	T	0.38	6,585	0.030 (0.013)	0.08	0.41	54,288	0.045 (0.006)	0.09	0.042 (0.006)	0.295	8.90
rs13329353	15	DET1	T	0.66	6,803	0.041 (0.013)	0.14	0.68	54,288	0.061 (0.007)	0.16	0.057 (0.006)	0.176	45.50
rs17767491	16	MAF	A	0.69	6,815	0.067 (0.013)	0.35	0.68	54,288	0.088 (0.007)	0.34	0.083 (0.006)	0.155	50.60
rs1042673	17	SOX9	A	0.52	6,472	−0.014 (0.013)	0.02	0.52	54,288	−0.055 (0.006)	0.14	−0.048 (0.006)	0.004	87.80
rs963384	17	SOX9	T	0.46	6,287	0.025 (0.013)	0.06	0.46	54,288	0.035 (0.006)	0.06	0.033 (0.006)	0.485	0.00
rs4804413	19	INSR	T	0.41	6,825	0.065 (0.012)	0.37	0.44	51,942	0.053 (0.006)	0.13	0.055 (0.006)	0.371	0.00
rs1203944	20	FOXA2	A	0.21	6,817	−0.043 (0.015)	0.12	0.23	54,288	−0.051 (0.007)	0.09	−0.050 (0.007)	0.629	0.00
rs6724073	2	DIRC3	T	0.74	6,405	0.035 (0.015)	0.09	0.74	52,047	0.051 (0.008)	0.09	0.047 (0.007)	0.347	0.00
rs28502438	3	TM4SF4	T	0.57	6,684	0.019 (0.013)	0.03	0.57	54,288	0.034 (0.006)	0.05	0.031 (0.006)	0.295	8.90
rs13100823	3	IGF2BP2	T	0.34	6,833	−0.040 (0.013)	0.13	0.31	54,288	−0.041 (0.007)	0.07	−0.041 (0.006)	0.946	0.00
rs59381142	3	HES1	A	0.23	6,482	−0.015 (0.015)	0.01	0.24	52,048	−0.058 (0.008)	0.12	−0.048 (0.007)	0.011	84.40
rs1265091	6	PSORS1C1	T	0.19	6,835	0.047 (0.016)	0.12	0.20	47,651	0.057 (0.009)	0.10	0.055 (0.008)	0.586	0.00
rs56009477	8	SLC25A37	A	0.83	6,819	0.048 (0.017)	0.12	0.84	54,288	0.052 (0.008)	0.07	0.051 (0.007)	0.831	0.00
rs10957494	8	SULF1	A	0.72	6,801	−0.034 (0.014)	0.09	0.69	54,288	−0.040 (0.007)	0.06	−0.039 (0.006)	0.701	0.00

Table 1 (continued)

SNP	Chr	Gene	Coded allele	GoDARTS				Prior association[1]				Meta-analysis		
				freq	N	β (SE)†	% var	freq	N	β (SE)†	% var	β (SE)	Het. p value	I²·%
rs118039499	8	TG	A	0.98	6,625	0.125 (0.045)	0.12	0.98	48,736	0.184 (0.024)	0.15	0.171 (0.021)	0.247	25.30
rs2739067	8	TG	A	0.61	6,828	-0.015 (0.013)	0.02	0.60	54,288	-0.042 (0.006)	0.08	-0.037 (0.005)	0.059	71.90
rs9298749	9	C9orf92	A	0.6	6,609	-0.033 (0.013)	0.1	0.59	54,288	-0.039 (0.006)	0.07	-0.038 (0.006)	0.675	0.00
rs11255790	10	GATA3	T	0.3	6,777	-0.040 (0.013)	0.12	0.30	54,288	-0.041 (0.007)	0.07	-0.041 (0.006)	0.946	0.00
rs4933466	10	PTEN	A	0.59	6,325	0.025 (0.013)	0.06	0.60	54,288	0.040 (0.006)	0.07	0.037 (0.006)	0.295	8.90
rs4445669	11	CADM1	T	0.43	6,828	-0.021 (0.012)	0.04	0.46	54,288	-0.040 (0.006)	0.07	-0.036 (0.005)	0.157	50.10
rs7329958	13	SPATA13	T	0.33	6,825	-0.030 (0.013)	0.08	0.35	54,288	-0.044 (0.007)	0.08	-0.041 (0.006)	0.343	0.00
rs11159482	14	TSHR	T	0.05	6,663	0.068 (0.028)	0.09	0.09	54,288	0.085 (0.013)	0.11	0.082 (0.012)	0.582	0.00
rs59334515	14	TSHR	T	0.24	6,819	-0.034 (0.014)	0.08	0.22	54,288	-0.054 (0.007)	0.10	-0.050 (0.006)	0.201	38.80
rs12893151	14	TSHR	A	0.2	6,726	-0.033 (0.015)	0.07	0.22	54,288	-0.062 (0.008)	0.13	-0.056 (0.007)	0.088	65.60
rs1045476	16	ADCY9	A	0.17	6,746	0.024 (0.016)	0.03	0.18	54,288	0.049 (0.008)	0.06	0.044 (0.007)	0.162	48.80
rs30227	16	MIR365A	T	0.6	6,827	-0.032 (0.013)	0.09	0.61	54,288	-0.047 (0.006)	0.10	-0.044 (0.005)	0.295	8.90
rs77819282	17	NSF	A	–	–	–	–	0.24	54,288	0.045 (0.007)	0.07	–	–	–
rs1157994	17	BCAS3	A	0.03	6,686	-0.080 (0.034)	0.08	0.05	50,232	-0.090 (0.016)	0.07	-0.088 (0.014)	0.79	0.00
rs12390237	X	PRKX	A	–	–	–	–	0.62	36,501	0.046 (0.007)	0.09	–	–	–

Chr, chromosome; Het. p, heterogeneity p value; SE, standard error; SNP, single-nucleotide polymorphism; TSH, thyroid-stimulating hormone; GoDARTS, Genetics of Diabetes and Audit Research Tayside Study. † Univariate linear regression models; β = effect size (mIU/L). N = number of subjects used for the association test. [1] Teumer A, Chaker L, Groeneweg S, Li Y, Di Munno C, Barbieri C, et al. Nature Communications. 2018;9:4455.

Table 2. Description of genotyped euthyroid patients with osteoporotic bone fractures (cases) and their comparison controls (n = 5,599)

Characteristic	Cases (n = 666)	Controls (n = 4,933)	p value
Gender – female, n (%)	428 (64.2)	1,758 (35.6)	<0.001
Diabetes mellitus, n (%)	646 (97.0)	4,796 (97.2)	0.740
Mean (SD)			
Age, years	64.4 (10.6)	58.5 (12.1)	<0.001
BMI, kg/m²	29.1 (5.2)	30.9 (5.6)	<0.001
Serum TSH, mU/L*	1.7 (1.2–2.3)	1.8 (1.3–2.4)	0.009

BMI, body mass index; TSH, thyroid-stimulating hormone. * Median (interquartile range) of measurements recorded throughout the study period.

TSH-raising alleles had increased serum TSH concentration (β = 0.67, 95% CI 0.61–0.73, $p = 2.0e{-}108$).

The TSH-GRS was associated with the risk of hypothyroidism in 6,759 individuals (1,153 cases and 5,606 controls) with an OR of 1.32 (95% CI 1.09–1.62, $p = 5.2e{-}03$), 1.56 (95% CI 1.28–1.89, $p = 8.8e{-}06$), and 1.98 (95% CI 1.63–2.40, $p = 2.2e{-}12$) for the second, third, and fourth quartiles, respectively, compared to the first quartile. Figure 2a shows the risk of hypothyroidism across the range of TSH-GRS by gender. Figure 2b shows the risk of osteoporotic bone fracture across the range of TSH-GRS by gender. Male carriers of greater numbers of TSH-raising alleles were at decreased risk of fractures.

The description of euthyroid patients with osteoporotic bone fractures (n = 666) and their comparison cohort (n = 4,933) is described in Table 2. Age-sex-adjusted serum TSH was inversely related to bone fractures with an OR of 0.68 (95% CI 0.55–0.84, $p = 3.8e{-}04$) for the highest tertile, where average TSH concentrations per tertile were 1.07, 1.77, and 2.71 mIU/L, respectively. MR analysis showed that compared with the lowest tertile, those in the highest tertile had an OR of 0.77 (95% CI 0.63–0.94, $p = 1.2e{-}02$). Men in the highest tertile had an OR of 0.59 (95% CI 0.42–0.83, $p = 2.6e{-}03$, n = 3,413), while women in a similar comparison had an OR of 0.93 (95% CI 0.71–1.21, $p = 0.61$, n = 2,193). As part of the sensitivity analyses, we repeated this MR analysis after removing SNPs with potential pleiotropic effects that might confound the relationship between TSH and osteoporotic bone fracture. Four variants associated with TSH were also found to be significantly associated with BMD: *SYN2*-rs1663070, *ABO*-rs8176645, *CADM1*-rs4445669, *ADCY9*-rs1045476) [16]. This modified analysis with a

TSH-GRS of 54 variants yielded a similar OR for bone fracture per unit increase in serum TSH (mIU/L) of 0.63 (95% CI 0.45–0.88, $p = 6.7e-03$, $n = 3,575$) for men in the highest tertile compared to the lowest, while no difference in a similar comparison was observed in women (OR = 0.99, 95% CI 0.81–1.37, $p = 6.8e-01$, $n = 2,292$).

Discussion

This record linkage study used electronic databases to validate genetic loci discovered in GWAS with hypothyroidism and serum TSH concentrations in a Scottish Caucasian population from the GoDARTS database. Using MR, this study showed a causal (inverse) link between serum TSH concentrations and osteoporotic bone fracture for men.

GoDARTS is a longitudinal cohort, and thus more than one serum TSH was available for the majority of participants (i.e., 8 measurements on average). Although the number of TSH measurements would not affect our results, the use of an average TSH reflects the TSH concentration better than a single measurement as in a cross-sectional study. The consistency of SNP effects on serum TSH of this study with the previously reported by Teumer et al. [3] was demonstrated, and any discrepancies seem to be explained mostly by differences in the way TSH measurement was done or by chance. Our study accounted for a larger serum TSH variation (11.6%) than that provided to us by Teumer et al. [3] (9.35%), but also than that reported by Taylor et al. [2] (7.1%) and Salem et al. [4] (5.8%). We acknowledge that the higher explained variance observed here could be because of some differences in sample size and/or allele frequency. However, the cohorts used for our study and those used by Teumer et al. [3] are of the same ethnicity-white European cohort, which is reflected in the similar allele frequencies observed across these 2 study populations. Thus, it seems unlikely that as the causal variants have still not been confirmed through functional studies, the Scottish population could have a different LD structure between these common SNPs and the causal variants. This finding in our cohort has been pointed out in a previous publication [10]. Like Teumer et al. [3], the TSH-GRS was associated with the risk of hypothyroidism in men and women, and it showed a higher risk for females.

The strength of the association between the TSH-GRS and serum TSH was confirmed by an *F*-statistic of 179, indicating that our instrument is strong and therefore unlikely to be susceptible to weak instrument bias [17].

Thus, under the assumption that this TSH-GRS is a valid instrument for serum TSH concentration, we estimated the causal effect of serum TSH on osteoporotic-related bone fractures using MR analyses. In our euthyroid cohort, serum TSH was significantly inversely related to osteoporotic bone fracture, but only male carriers of greater numbers of TSH-raising alleles were at decreased risk. Although women showed a higher risk of osteoporotic bone fractures, there was not a significant difference between carriers of greater numbers of TSH-raising alleles (highest tertile) and lower carriers. We observed a very small nonsignificant protective effect for the highest tertile in women (OR = 0.93, $p = 0.61$).

Serum TSH concentrations have been associated with bone fractures in published observational studies [7, 18–20]. These studies reported that lower concentrations of TSH were associated with an increased risk of osteoporosis and fractures [19]. Data on women from these studies were mostly from healthy post-menopausal females (i.e., group at highest risk) but also from younger women [7, 19]. However, women in our study cohort were older than 58 years on average (i.e., many with post-menopausal status) and the MR analysis showed their risk of osteoporotic bone fractures was independent of serum TSH within the normal range. Although we still do not know the underlying mechanisms for sex differences in our results, we hypothesize that it could be related to menopausal changes that happen in women but not in men. Menopausal changes are likely to have a greater impact on osteoporotic bone fracture risk than variation in normal thyroid function in women. Thus, changes in estrogen status may be hiding the impact on fractures of differences in serum TSH in women that were observed in men. It is also possible that given that some TSH-increasing alleles showed a different impact on serum TSH variability in women compared to men [1, 21], unknown gender-specific effects of TSH could help to explain our findings as well.

In order to improve the reliability of our MR results and to ensure that there were no obvious pleiotropic SNPs in the GRS, we performed a sensitivity analyses by excluding potential pleiotropic variants. Thus, we repeated the MR analysis after removing SNPs with horizontal pleiotropic effects that might confound the relationship between TSH and osteoporotic bone fractures. The associations of the genetic variants used as genetic instruments with BMD were annotated using the PhenoScanner database. Four variants in chromosomes 3, 9, 11, and 16 were significantly associated with BMD and thus removed from the GRS. This additional MR analysis yielded similar results. Other potential confounders that could have been considered

Soto-Pedre/Siddiqui/Mordi/Maroteau/
Soto-Hernaez/Palmer/Pearson/Leese

(i.e., diabetes mellitus, body mass index, or smoking) would mostly relate to bone fracture through their effect on BMD as well [22, 23]. Obviously, we can never exclude unknown pleiotropy, but having similar results after exclusion of these variants should at least address this concern.

We acknowledge that a nonlinear model does not guarantee that the residuals from the second-stage regression are uncorrelated with the instruments in a one-sample MR with a dichotomous outcome. However, the 2-stage estimator with a logistic second-stage model still provides a valid test of the null hypothesis [13]. We also acknowledge that a 2-sample MR would have been better to deal with potential pleiotropy, but we did not have available an additional sample from the same population with individual-level data.

In conclusion, we have shown that EMR-linked genomic data allowed replication of previously identified SNPs associated with several thyroid-related traits without additional genotyping costs. This study provided information that genetically raised serum TSH concentrations are causally associated with decreased osteoporotic bone fracture risk in men, but not in women. Our results also suggest potential benefits for monitoring TSH in euthyroid men who may be at particular risk of avoidable bone fractures and implications for fracture risk stratification.

Statement of Ethics

All analyses were performed on anonymized datasets. The study was approved by the East of Scotland Research Ethics Service-Eo-SRES (HIC datasets V2, REC ref. 18/ES/0126, IRAS ID 143637), and informed consent had been obtained for all participants.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Author Contributions

E.S.-P. planned the study, researched/analysed data, and wrote the manuscript. M.K.S. and C.M. researched data and contributed to discussion. J.S.-H. researched data. C.N.P., E.R.P., and I.M. contributed to data analysis and to discussion. G.P.L. planned the study, researched data, contributed to the discussion, and reviewed/edited the manuscript.

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