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Genome-wide analysis of thyroid function in Australian adolescents highlights SERPINA7 and NCOA3

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The authors have no conflicts of interest to declare.

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ABSTRACT

Objective

Genetic factors underpin the narrow intraindividual variability of thyroid function, although precise contributions of environmental versus genetic factors remain uncertain. We sought to clarify the heritability of thyroid function traits and thyroid peroxidase antibody (TPOAb) positivity and identify single nucleotide polymorphisms (SNPs) contributing to the trait variance.

Methods

Heritability of thyroid stimulating hormone (TSH), free T4 (fT4), free T3 (fT3) and TPOAb in a cohort of 2854 euthyroid, dizygous and monozygous twins (age range 11.9-16.9 years) from the Brisbane Longitudinal Twin Study (BLTS) was assessed using structural equation modelling. A genome-wide analysis was conducted on 2832 of these individuals across 7,522,526 single nucleotide polymorphisms as well as gene-based association analyses. Replication analysis of the association results was performed in the Raine Study (n= 1115) followed by meta-analysis to maximise power for discovery.

Results

Heritability of thyroid function parameters in the BLTS was 70.8% (95% CI: 66.7-74.9%) for TSH, 67.5% (59.8-75.3%) for fT4, 59.7% (54.4-65.0%) for fT3 and 48.8% (40.6-56.9%) for TPOAb. The genome-wide association study (GWAS) in the discovery cohort identified a novel association between rs2026401 upstream of *NCOA3* and TPOAb. GWAS meta-analysis found associations between TPOAb and rs445219, also near *NCOA3*, and fT3 and

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rs12687280 near *SERPINA7*. Gene-based association analysis highlighted *SERPINA7* for fT3 and *NPAS3* for fT4.

Conclusion

Our findings resolve former contention regarding heritability estimates of thyroid function traits and TPOAb positivity. GWAS and gene-based association analysis identified variants accounting for a component of this heritability.

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Introduction

The relatively broad reference range for thyroid function parameters is reflective of the variability that exists at a population level. Within individuals, however, thyroid stimulating hormone (TSH), free T3 (fT3) and free T4 (fT4) are typically regulated in a narrower range, reflecting a unique physiological set-point for pituitary thyroid axis function [1]. While the presence of a strong genetic component to this set-point has been established from twin and family studies, the precise extent to which it is governed by genetic versus environmental factors remains uncertain, particularly for fT3 and fT4 where previous estimates vary substantially.

With the exception of Samollow et al. [2], familial studies have shown a consistently large genetic contribution to TSH variance [3-5]. Panicker et al. [4] and Hansen et al. [5] comprise two large-cohort twin studies that have explored heritability using structural equation modelling (SEM) in healthy euthyroid individuals involving 2124 subjects from the United Kingdom and 1380 subjects from Denmark. Although both studies provided similar estimates for TSH heritability (64% and 65%), Panicker et al. determined a lower heritability of serum thyroid hormone concentrations (fT3 23%, fT4 39%) compared to Hansen et al. (fT3 64%, fT4 65%). TPOAb heritability has previously been reported as 69% [6] and 73% [7], the former figure from a study of individuals with type 1 diabetes; arguably at higher risk of autoimmunity. As such, further familial studies are needed to elucidate the comparative contributions of genetic and environmental factors in determining thyroid function.

Following the observation of a substantial heritable component in thyroid function setpoints, genome wide association studies (GWAS) have identified numerous single

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nucleotide polymorphisms (SNP) associated with thyroid function parameters [8-12]. These GWAS have been able to account for between 1 and 5% of the phenotypic variance of serum TSH [8-10] and 2.3% of fT4 [10]. A meta-analysis by Taylor et al. [11] accounted for a much greater proportion of variance in a large whole genome sequence-based analysis, with >20% of the TSH and fT4 variance attributed to identified SNPs. Teumer et al. [13] found that 33% of TSH and 21% of fT4 variance was attributable to both common and infrequent alleles with a minor allele frequency (MAF) >1%. However, a significant proportion of heritability remains unaccounted for, which may indicate the presence of lower frequency variants with large effect sizes that are yet to be discovered [11].

The Brisbane Longitudinal Twin Study (BLTS) is a large epidemiological cohort of twins, recruited from adolescence and studied longitudinally. This represents an ideal cohort with which to further examine the heritability of thyroid function and perform a GWAS to delineate new, and validate existing, data on polymorphisms potentially accounting for observed variance in thyroid phenotypes. In this study, analysis of thyroid function (TSH, fT4 and fT3) and TPOAb status was undertaken in the cohort of monozygotic (MZ) and dizygotic (DZ) twins from the BLTS, with subsequent replication in an independent cohort. Finally, a meta-analysis was undertaken to maximise the discovery potential of the data. In doing so, we aimed to clarify the heritability of thyroid function parameters by determining if estimates of heritability made in adolescence are consistent with those made in infancy and adulthood. Secondly, we sought to identify novel loci using GWAS relevant to thyroid function variability.

Participants and Methods

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Participants

Discovery study participants were part of the BLTS conducted at the Queensland Institute of Medical Research (QIMR) Berghofer, Australia [14]. The BLTS began in 1992, with approximately 100 pairs of 12-year-old twins (alongside their non-twin siblings and parents) with additional participants recruited annually thereafter, gathering a range of behavioural, social, anthropometric, biochemical and genetic data until 2016. After recruitment, follow-up occurred at ages 14, 16, 19 and 25. The BLTS was approved by the Human Research Ethics Committee of the QIMR (P193, P455) and participants provided written consent upon study enrolment. The present study used longitudinal data on thyroid function parameters from 5072 observations on 2854 MZ and DZ twins. Twelve participants were excluded on the basis of strong evidence of thyroid dysfunction, namely serum TSH >10 or <0.1 mU/L, or with TSH >6 mU/L accompanied by positive TPOAb.

The Raine Study is a longitudinal multigenerational study and was used for replication and in the meta-analysis with the BLTS. The Raine Study consists of a cohort of women from Perth, Western Australia, recruited during pregnancy at 16-20 weeks gestation between 1989 and 1991, and their offspring (Generation 2) who have been followed since birth as detailed previously [15]. Approval to conduct the Raine Study was obtained from the Human Ethics Committee of the University of Western Australia (RA/4/20/5722). Written consent was obtained for all participants. Thyroid function results and genetic data from Generation 2 participants at age 14 were used in this study.

Biochemistry methods

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In the Raine Study, fasting morning blood samples measuring serum TSH, fT4, fT3 and TPOAb were collected. In the BLTS, blood sample timing was not uniform and participants were not fasted. Analytes were measured by chemiluminescent immunoassay on an Abbott ARCHITECT analyser (Abbott Diagnostic, Illinois, USA) as described previously [16]. A TPOAb titre of >6kU/L was considered positive.

Statistical analysis

TSH and TPOAb values were natural log transformed to normalise the distribution before further analysis. After clinical exclusions, participants with a measurement >4 standard deviations from the mean for TSH, fT3 or fT4 were also excluded. For TPOAb, all subjects were retained for analysis. Using linear regression, thyroid traits were adjusted for the covariates age, sex, BMI, batch and reagent lot, with resulting residuals used in subsequent analysis. TSH, fT3 and fT4 were also adjusted for TPOAb status. For the heritability study, subjects were first categorised by age: 12 (11.9-12.9), 14 (13.9-14.9) or 16 (15.9-16.9) years old. Analysis of thyroid function phenotypes using samples taken at the last visit for each twin pair was then undertaken to maximise statistical power. For each trait and age category, intraclass correlations were calculated within the MZ and DZ groups (r_{MZ}, r_{DZ}). The magnitude of each correlation was tested for difference from zero, applying an adjusted significance threshold of $\alpha'=0.05/8=0.006$, and using Fisher's Z-transformation, the difference between each pair of correlations, $r_{MZ}-r_{DZ}$, was also assessed for significance (against $\alpha'=0.05/4=0.013$).

Twin data and model fitting analysis

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The classic twin model examines the variance in phenotypes in MZ and DZ twins, and using knowledge of their genetic similarities (DZ twins share approximately half the genotype of MZ twins), determines the relative genetic and environmental contributions to this variation [17]. SEM is used to determine the components of trait variance attributable to additive genetic (A), non-additive genetic (D); common (C) and unique environmental (E) effects. This was undertaken in the BLTS cohort. Relative contributions of these factors were tested using a maximum likelihood approach, comparing fully specified ACE or ADE models with restricted submodels (AE, CE, E). For each phenotype, the model with the lowest Akaike information criterion (AIC) value was selected as the best fit and compared with other candidate models via a likelihood ratio test (LRT). A simpler model with fewer parameters was selected as optimal if LRT showed no loss of fit. Analysis was performed in the R statistical computing environment, version 3.6.0, with the additional packages *OpenMx* version 2.6.7 [18] and *umx* incorporating the umxSexLim function [19].

Genome-wide association analysis

Genotype data for the discovery study were obtained using either the HumanCoreExome-12v1.0 or IlluminaHuman610WQuad bead chip. Genotyping and quality control procedures for the BLTS are described elsewhere [20]. SNP coordinates were determined using human genome Build37 (hg19). The significance threshold for the association test was p=5E-8 for GWAS variants and p=1E-5 for suggestive association. Genetic data from 2832 individuals were available and after imputation and quality control, 7,522,526 SNPs were analysed. Data were imputed to the Haplotype Reference Consortium r1.1 reference panel [21]. Following principal components analysis of SNP data using the SMARTPCA program (version 16000) in EIGENSTRAT [22], individuals lying >6SD outside the European mean for PC1

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and/or PC2 were excluded to ensure homogenous ancestry in the sample. Association testing of thyroid phenotypes and genetic variants was performed using a linear mixed model to account for related subjects, implemented in the GEMMA (Genome-Wide Efficient Mixed Model Association) software package [23]. Prior to testing, traits were adjusted for covariates and distributions were normalised using the rank-based inverse normal transformation. Conditional analysis was performed on the results for genome-wide significant loci using GCTA [24] referencing the lead SNP found on primary analysis, and applying the same genome-wide significance threshold (p=5E-8), to assess independence of SNPs from the lead variant. The Raine Study was genotyped using the Illumina 660W Quad BeadChip. Replication was reached where BLTS GWAS variants achieved the multiple testing corrected threshold of p<0.017 in the Raine Study.

Meta-analysis of GWAS data

Meta-analysis of the GWAS data using the two cohorts was conducted using METAL software [25], which weights each effect size estimate with the inverse of its corresponding standard error. Results from conditional analyses were similarly meta-analysed. Genome-wide significant and suggestive thresholds for the single-point analyses were set at p=5E-8 and p=1E-5, respectively.

Gene-Based Association Analysis

GWAS meta-analysis results were analysed to give an aggregated gene-based association result using VEGAS2v2 software [26]. The analysis window included the open reading frame of each gene and 50kb of upstream and downstream sequence, enabling capture of variants in regulatory regions and non-coding regulatory variants. A value of p<1.9E-6 was selected

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as significant for the gene-wide association analysis (suggestive association threshold was *p*=1E-5), reflecting a multiple testing correction for the 26,056 genes analysed.

Bioinformatics analysis

Analysis of the linkage disequilibrium (LD) surrounding variants of interest was performed using LDlink (1000GP Phase 3 EUR population) [27] and HaploReg v4.1 [28]. Prediction of histone marks, DNAse hypersensitivity sites and expression quantitative trait locus associations was performed using HaploReg v4.1 [28] and genomic evolutionary rate profiling scores were obtained using genome-wide annotation of variants (GWAVA) [29].

Results

Descriptive statistics

Summary data for thyroid parameters of BLTS and Raine Study participants are presented in Table 1. In the BLTS cohort, median TSH (2.5^{th}-97.5^{th} centile) was 1.44mU/L (0.56-3.37), and in the Raine Study, 1.90mU/L (0.74-4.58). TPOAb was positive in 4.1% and 4.2% of the BLTS and Raine cohorts, respectively.

Intraclass correlations

Intraclass correlations for covariate-adjusted fT3, fT4, TSH and TPOAb status for BLTS are presented in Supplemental Table 1. All thyroid function parameters demonstrated a significantly greater non-zero within-pair correlation in MZ compared with DZ twins (ratios ranged from 1.48 to 4.23; all $p<0.0013$), suggesting substantial heritability.

Biometric analysis

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Biometric analysis results are presented in Table 2. Mean heritability estimates were as follows: fT4 67.5%, fT3 59.7%, TSH 70.8%and TPOAb 48.8%. A model consisting of additive genetic (A) and unique environmental (E) effects provided best fit for thyroid function phenotypes using samples taken at last visit across all ages (mean 14.8 years, SD 1.5). Heritability differed between age groups for some thyroid phenotypes, including lower heritability for TSH at ages 12 and 14 (45.3% and 34.2%, respectively) and fT3 at age 16 (A=20.6%).

GWAS – Discovery study

In the discovery cohort, 28 TSH-related SNPs reached genome-wide significance (p<5E-8). All SNPs were of similar effect size and located upstream of *FOXE1,* within a 26.2kb region on chromosome 9. When conditioned for the lead SNP, rs10739496, the remaining SNPs were not independently significant (Table 3). TPOAb positivity was significantly associated with 2 SNPs, both located in a noncoding region on chromosome 20 approximately 100kb upstream of *NCOA3*, a gene encoding for nuclear receptor coactivators. Given the identical effect size and p-value of these two SNPs, conditional analysis was undertaken with rs2026401 arbitrarily selected as the top SNP, confirming the second SNP was not independently significant. No SNPs or genes associated with fT3 or fT4 reached statistical significance.

GWAS – Replication study

The TSH-associated SNP, rs10739496, achieved replication (p=3.54E-4). For TPOAb, rs2026401 was not replicated (Table 3).

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GWAS – Meta-analysis

Meta-analysis results are presented in Table 4. Figure 1 depicts the Miami plots for all SNPs associated with TSH, fT4, fT3 and TPOAb. Quantile-quantile plots (Supplemental Figure 1) did not identify any significant confounding effects (such as population stratification) for thyroid phenotypes. The main findings of the meta-analysis were novel associations between fT3 and Xq22.3 near *SERPINA7*, TPOAb and 20q13.12 near *NCOA3* (Figure 2) and validation of known associations between TSH and both *VEGFA* and *FOXE1* (Supplemental Figure 2).

Analysis of the combined cohorts revealed 27 SNPs located on Xq22.3 near *SERPINA7*, a gene encoding thyroxine binding globulin (TBG). When conditioning for the top hit, rs12687280 (p=1.86E-11), the remaining variants were not independently significant.

There were 39 TPOAb SNPs reaching genome-wide significance. These variants were associated with *NCOA3*. Conditional analysis confirmed that these were in LD with rs445219, the top hit. The *NCOA3*-associated TPOAb variant from the discovery cohort, rs2026401 reached suggestive association on meta-analysis (p=7.27E-08).

In total, 75 TSH-associated variants were found on meta-analysis. The lead variant on chromosome 9 was rs1561961 (an intronic variant of *TRMO*), which was in LD with the remaining 69 SNPs at this location and distinct from the TSH-associated variants encountered in the discovery cohort. The remaining 5 TSH-associated SNPs were located on chromosome 6 near *VEGFA* with the top hit, rs11755845, in LD with the remaining variants.

Gene-based analyses

Gene-based studies of the meta-analysis data (Table 5) for TSH highlighted *FOXE1*, *TRMO and HEMGN* as significantly associated (p<1.9E-6); all three genes are clustered together at

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9q22.33 with *HEMGN* probably only featuring due to its close proximity to *TRMO*. Gene-based analysis of fT4 highlighted *NPAS3* as significant, with *SERPINA7* and *DIO1* notable among the suggestive associations. For fT3, a significant association was observed with *SERPINA7*.

For TPOAb there were no significant associations, however, *NCOA3* was among suggestive associations (*p*=5.0E-6).

Discussion

Heritability

In this heritability analysis of euthyroid individuals enrolled in the BLTS, we confirmed the presence of a significant genetic component to the phenotypic variance of TPOAb positivity as well as fT4, fT3, and TSH concentrations. The genetic contribution to fT4 variance was concordant with the 65% heritability reported by Hansen et al. [5] as opposed to the lower estimates in other studies [3-4]. The environmental contribution to variance was greater within certain subgroups, namely TSH in 12 and 14-year old and fT3 in 16-year old subjects. The precise environmental factors accounting for these differences are unclear, although some moiety may reflect complex sexual maturation effects and interactions. Environmental factors, including socioeconomic conditions and nutrition, are known to strongly affect timing of menarche [30] and male puberty [31], and dynamic endocrine changes in the anterior pituitary through puberty and adolescence to adulthood may extend to effects on pituitary setpoints for TSH and thyroid hormones.

TPOAb heritability was 48.8%, which is lower than the 69-73% reported previously [6-7]. Wang et al. [6] examined individuals with type 1 diabetes and their twins, which may

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comprise a cohort more predisposed to autoimmunity, including greater TPOAb prevalence, although euthyroid twins were the subject of Hansen et al. [7] where TPOAb heritability was 73%. The relatively high environmental contribution to TPOAb variance in our cohort could reflect the importance of environmental influences on development of TPOAb in younger individuals, supporting a hypothesis of a multifactorial nature to TPOAb heritability. A recent study suggested that the heritability of Hashimoto's disease may be greater in men than in women [32]. Our data on TPOAb levels appear consistent with that, with a higher proportion of variance due to additive genetic effects in males versus females at 16 years. Notably, there was a stronger genetic component to TSH variance in females (A=71.4%) within the 16-year-old group compared to males (A=65.1%). This could reflect gender-based differences in the effect size of thyroid-associated genes (such as *PDE8B*, *PDE10A*), the presence of sex-specific loci (such as *NETO1*/*FBXO15*) or indeed the influence of sex hormones and the association of female gender with thyroid autoimmunity [10].

Previous heritability studies have examined predominantly adult cohorts, making direct comparisons with our study difficult. Our findings suggest a greater effect of environmental factors during adolescence on thyroid phenotypes compared to older individuals. While data were adjusted for BMI, TPOAb positivity, batch, age and sex, perhaps other influences such as cigarette smoking [33], pubertal stage [34] or epigenetic differences [35] contributed to our findings.

Overall, the heritability findings from a large, well characterised cohort reinforce the existence of a genetically determined individual set-point for thyroid function and should contribute substantially to resolving previous contention over the precise magnitude of heritability for these clinically relevant thyroid traits.

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GWAS

Meta-analysis identified a novel and statistically significant association between fT3 and rs12687280, upstream of the gene encoding TBG, *SERPINA7*, on the X chromosome (Xq22.2). This association was further supported by gene-based analysis. TBG is the major thyroid hormone transport protein in serum. TBG variants, while not causative of thyroid disease, manifest with altered serum thyroid hormone levels including raised fT3 and raised or suppressed fT4 in euthyroid individuals as a consequence of altered thyroid hormone binding [36]. Multiple TBG variants exist, with some caused by single nucleotide substitution resulting in complete or partial TBG deficiency [36], the latter manifesting with reduced serum TBG levels or reduced thyroxine or triiodothyronine binding affinity. As rs12687280 is located upstream of *SERPINA7*, it may function, or be in LD with a variant that functions, as a transcriptional regulator for this gene. Should rs12687280 influence fT3 variability by altering TBG production, it is unclear why fT4 variability is unaffected. Notably, on gene-based analysis, *SERPINA7* reached a suggestive association with fT4. Furthermore, a metabolomics study in humans has previously highlighted an association between plasma thyroxine and rs7883218, within *SERPINA7* [37]. Further investigation using detailed molecular and functional studies may provide insight into the mechanism by which this variant alters TBG production or function.

Meta-analysis also demonstrated a novel association linking TPOAb with rs445219 near *NCOA3*. Two *NCOA3*-associated SNPs were identified in the discovery cohort, which reached suggestive association in the meta-analysis. *NCOA3* produces nuclear receptor coactivators which act as scaffolds, forming multi-subunit complexes that promote gene transcription in a hormone-dependent manner [38]. Unsurprisingly, *NCOA3* is implicated in oncogenic pathways for a range of malignancies [38]. Although the present study is the first to link

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TPOAb to *NCOA3*, its previous associations with systemic lupus erythematosus [39-40], psoriasis [39] and systemic sclerosis [41] are supportive of a role in autoimmunity. Indeed SRC3, a product of *NCOA3*, is highly expressed in B and T lymphocytes, particularly regulatory T cells (Treg) and is understood to downregulate immune responses [42].While *NCOA3* could be associated with a loss of self-tolerance linked to Treg dysfunction or aberrant lymphocyte function, the precise mechanism by which it influences TPOAb positivity is unclear at this time.

Gene-based analyses identified a novel association between *NPAS3* and fT4. *NPAS3* (neuronal PAS domain 3) encodes a transcription factor predominantly expressed in the central nervous system and implicated in neurodevelopmental and psychiatric disorders including Bipolar Disorder (BD) and Schizophrenia [43]. Whilst no previous studies have linked *NPAS3* with thyroid parameters, there is evidence of pleiotropy regarding loci implicated in both psychiatric disorders and metabolic traits. A whole-genome sequence study identified regions with shared associations between BD and thyroid function and thyroid antibodies using polygenic risk scores. The region harbouring *NPAS3*, 14q12-13.1, although not previously linked to thyroid traits, was associated with HDL cholesterol and BD [44]. Our findings associating *NPAS3* with fT4, given its role encoding a transcription factor, suggests that it may additionally serve as a regulatory protein in the HPA axis.

GWAS identified rs10739496 as significantly associated with TSH variability in the discovery cohort, and rs1561961 on meta-analysis, which are located on 9q22.33 in LD blocks upstream and downstream, respectively, of *FOXE1* (forkhead box protein E1). *FOXE1*,

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HEGMN and *TRMO* all reached significance on gene-based analysis, sharing rs1561961 as the associated top SNP, which reflects their co-localisation on 9q22.33 [45]. *FOXE1* has the most well characterised association with thyroid function: it encodes a protein critical to thyroid function, is implicated in TSH variation, hypothyroidism [12, 46] and thyroid cancer susceptibility [47], with oncogenesis promoted by its inactivation [48].

The association of *VEGFA* with rs11755845 and TSH is well established [9-10]. *VEGFA* regulates hypothalamic feedback by promoting conversion of T4 to T3, consequently suppressing release of TSH [49].

Strengths of the present study include cohort size, younger age (given our focus on normal thyroid physiology) and validation of GWAS results against an external cohort. Regarding limitations, restricting our analyses to individuals of European heritage could have obscured genetic associations that may have otherwise been evident in a cohort of greater ancestral diversity, such as the general Australian population. Timing of blood sampling in BLTS was not standardised, therefore circadian variation in thyroid hormones was unable to be accounted for.

In conclusion, a heritability study of a large euthyroid twin cohort clarified the significant genetic component to thyroid function variability and TPOAb positivity consistent with the higher range of estimates seen in previous studies. The GWAS meta-analysis identified novel variants linking fT3 with *SERPINA7* on Xq22.2 and TPOAb with *NCOA3* on 20q13.12. Gene-based analyses highlighted *SERPINA7* and *NPAS3* as additional contributors to variability of fT3 and fT4, respectively.

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Supplementary data

This is linked to the online version of the paper at [To be confirmed]

Disclosure

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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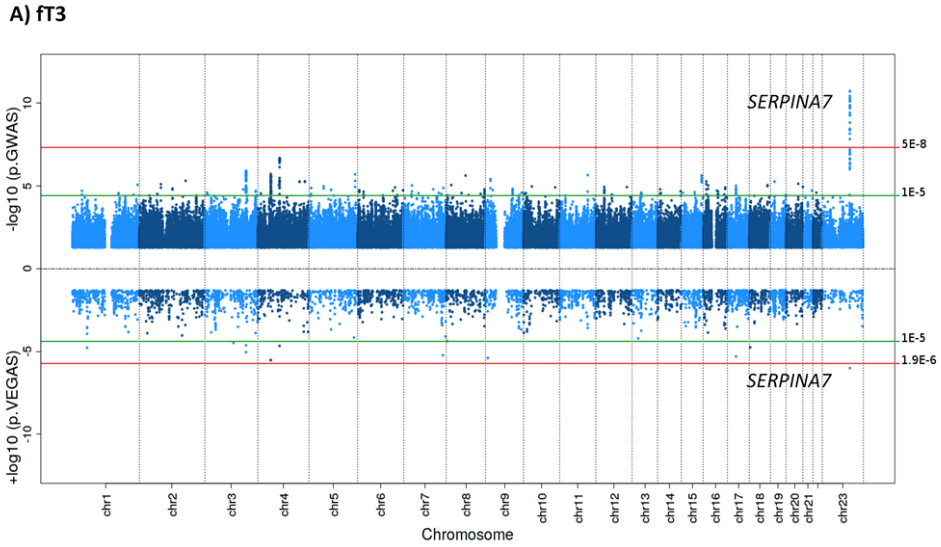

Figure 1. Miami plots showing genome-wide SNP (upper panel) and gene-based (lower panel) association results for thyroid function, A) fT3, B) fT4, C) TSH and D) TPOAb. Genome-wide significance thresholds for the SNP and gene-based association studies are p=5 E-8 and p=1.9 E-6 respectively (red). Suggestive thresholds for the SNP and gene-based association analyses are p=1 E-5 and are shown in green.

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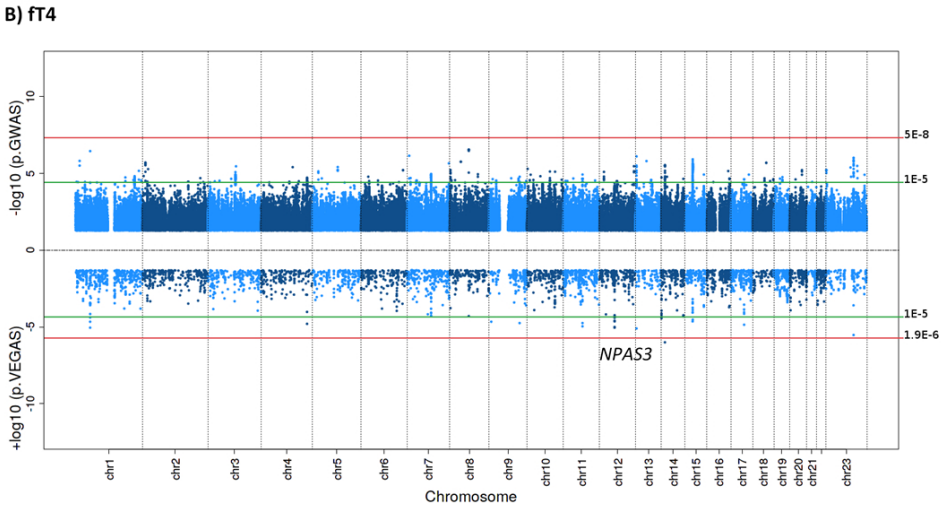

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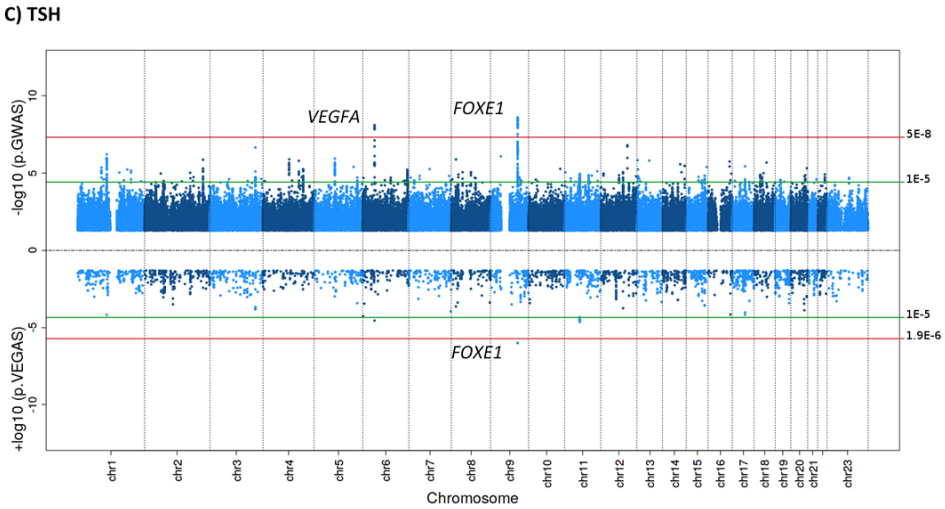

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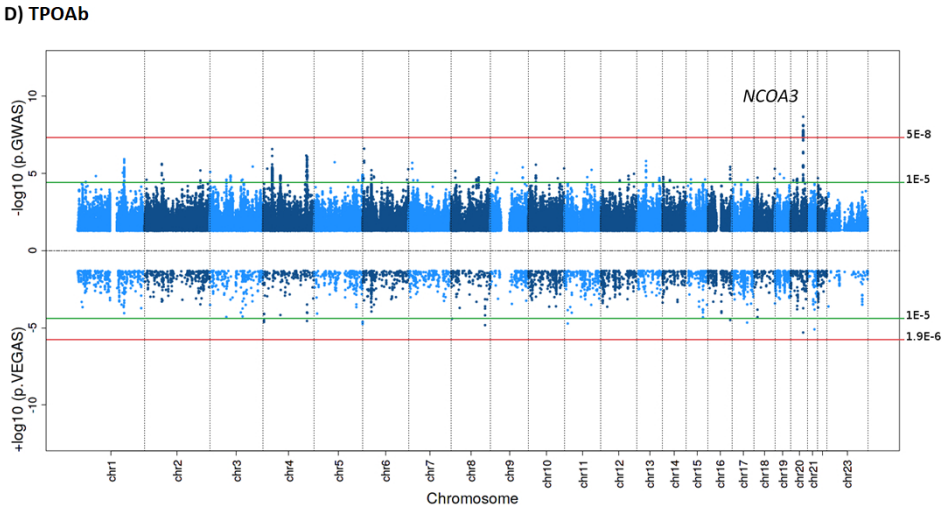

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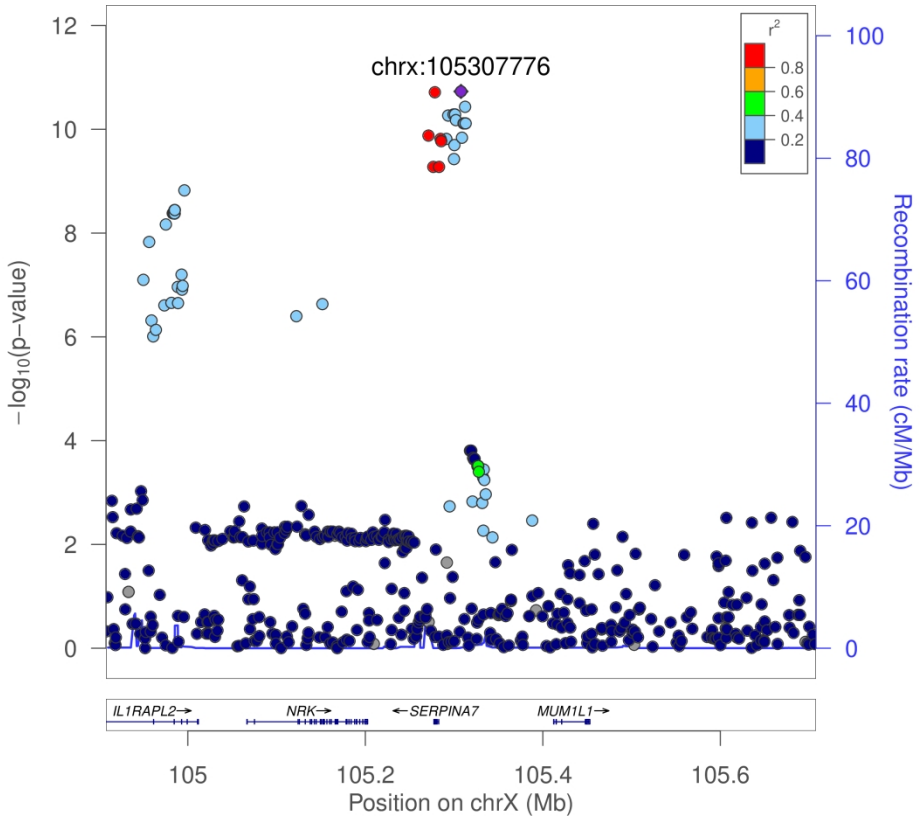

Figure 2. Regional association plot for meta-analysis results. A) fT3 at the Xp22.3 locus and B) TPOAb at the 20q13.12 locus. Genetic variants within 400kb of the lead variant are plotted by location on the x axis, with -log10 p value on the y axis. Colour fill of each data point reflects the LD (r2) with the lead variant (1000GP Nov 2014 EUR population). The position of genes in the locus (hg19) and recombination rate (blue line) is also indicated.

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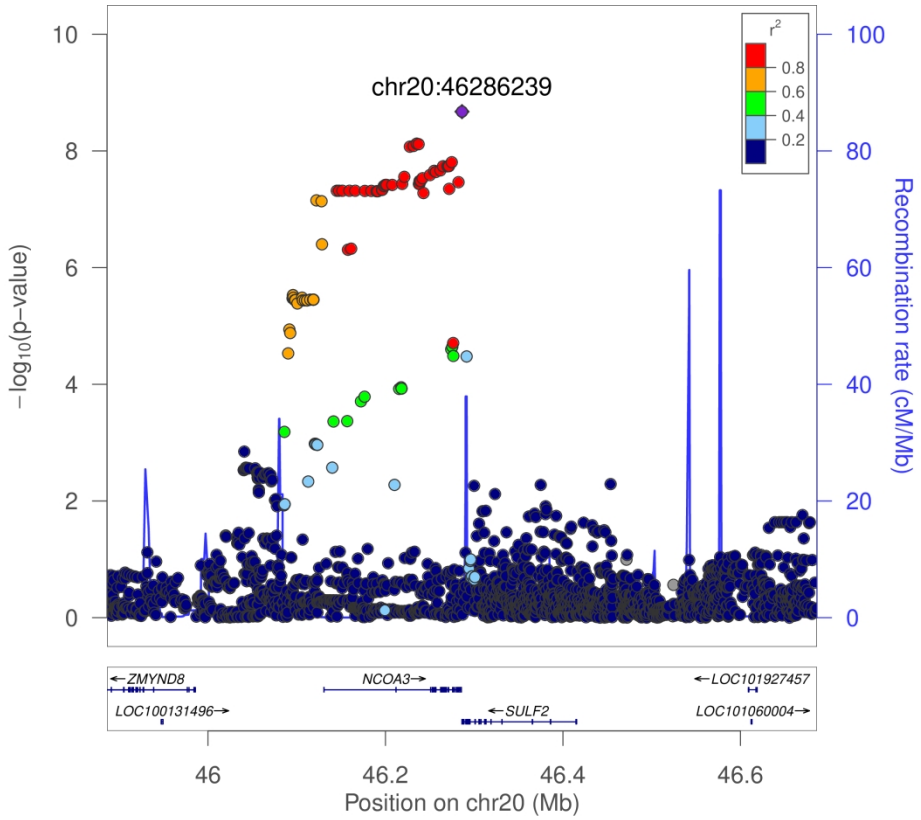

As per Figure 2A. (multipanel figure)

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Table 1 Descriptive data for thyroid parameters in the BLTS and Raine Study cohorts. Summary table represents values from the last visit of all subjects meeting inclusion criteria. Data are presented mean±S.D., *n* (%) or as median (2.5^{th}-97.5^{th} quartiles).

	n	**Age** (years)	**fT3** (pmol/L)	**fT4** (pmol/L)	**TSH** (mU/L)	**TPOAb positive**
Brisbane Longitudinal Twin Study						
All	2854	14.9±1.9	5.04±0.62	12.9± 1.34	1.44 (0.56, 3.37)	116 (4.1%)
Female	1508 (53%)	15.0± 1.9	4.82±0.59	12.9± 1.26	1.34 (0.52, 3.18)	94 (6.2%)
Male	1346 (47%)	14.8± 1.8	5.29±0.56	13.0±1.41	1.56 (0.63, 3.54)	22 (1.6%)
Raine Study						
All	1104	14.1±0.20	5.5±0.58	12.3± 1.27	1.90 (0.74, 4.58)	46 (4.2%)
Female	527 (48%)	14.1± 0.19	5.2±0.49	12.3±1.21	1.85 (0.72, 4.62)	36 (6.8%)
Male	577 (52%)	14.1±0.20	5.8±0.51	12.1±1.31	1.93 (0.82, 4.44)	10 (1.7%)

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Table 2 Structural equation modelling outcomes for thyroid parameters in the Brisbane Longitudinal Twin Study. Model of best fit is presented for each analyte. Proportions of variance (95% CI) represent additive genetic (A), common environmental (C) and unique environmental (E) contributions to phenotypic variation. 'Last' includes the observation from the most recent visit of every individual and therefore maximises the available sample for study.

Age	Model	Proportion of Variance (%)			DF	AIC	$\Delta\chi^2$	ΔDF	*P*-value
		A	C	E					
fT3									
12	AE	54.8 (45.5, 64.1)	-	45.2 (38.6, 51.8)	1527	4225.2	0.0	1	1
14	AE	61.1 (54.6, 67.6)	-	38.9 (32.4, 45.4)	1422	3874.9	0.3	1	0.585
16	ACE[a]	20.6 (-1.4, 42.6)	26.6 (8.6, 44.6)	52.7 (42.7, 62.7)	875	2413.3			
Last	AE	59.7 (54.4, 65.0)	-	40.3 (35.0, 45.6)	2084	5704.8	1.8	1	0.184
fT4									
12	AE	67.6 (58.6, 76.6)	-	32.5 (27.5, 37.5)	1527	4092.3	0.2	1	0.666
14	AE	63.9 (54.3, 73.6)	-	36.1 (30.2, 42.0)	1423	3871.5	0.4	1	0.525[b]
16	AE	67.4 (55.6, 79.2)	-	32.6 (26.0, 39.2)	877	2382.7	0.0	1	0.953
Last	AE	67.5 (59.8, 75.3)	-	32.5 (28.1, 36.9)	2085	5593.1	0.0	1	1
TSH									
12	ACE	45.3 (28.4, 62.1)	22.5 (7.9, 37.2)	32.2 (26.9, 37.6)	1526	4053.7			
14	ACE[a]	34.2 (13.4, 55.0)	37.4 (19.2, 55.6)	28.4 (22.7, 34.1)	1421	3809.0			
16	AE[c]								
	Female	71.4 (61.6, 81.2)	-	28.6 (18.8, 38.4)	873	2373.2	1.5	2	0.481[b]
	Male	65.1 (54.3, 75.9)	-	34.9 (24.1, 45.7)					
Last	AE	70.8 (66.7, 74.9)	-	29.2 (25.1, 33.3)	2084	5614.5	1.9	1	0.172
TPOAb									
12	AE	36.6 (26.7, 46.4)	-	63.4 (54.7, 72.2)	1539	4344.2	0.44	1	0.505[b]
14	AE	49.6 (40.0, 59.3)	-	50.3 (42.8, 57.7)	1442	4001.8	2.3	1	0.130
16	AE[c]								
	Female	40.3 (20.3, 60.3)	-	59.7 (39.7, 79.7)	886	2501.5	4.5	2	0.106[b]
	Male	56.4 (44.2, 68.6)	-	43.6 (31.4, 55.8)					
Last	AE	48.8 (40.6, 56.9)	-	51.2 (44.8, 57.7)	2101	5849.6	0.0	1	1

DF = degrees of freedom; AIC = Akaike information criterion; $\Delta\chi^2$ = difference in test statistic between the given model and the more complex model compared with; ΔDF = difference in degrees of freedom between the given model and the more complex model;
p-value from LRT comparing the 2 models. [a] Models with different means for male and female. [b] When compared with ADE model. [c] Models with different variance components for male and female.

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Table 3. SNPs in the discovery cohort reaching statistical significance on GWAS and results from the replication cohort.

Phenotype						Discovery			Replication		
	Chr	SNP	Position	Nearest gene	A1/A2	Freq A1	Effect	P	Freq A1	Effect	P
TSH											
	9	rs10739496	100552559	*FOXE1*	C/T	0.316	-0.2034	6.13E-10	0.334	-0.0315	3.54E-4
TPOAb											
	20	rs2026401	46122284	*NCOA3*	C/T	0.062	0.0663	2.72E-08	0.068	0.0243	1.58E-01
	20	rs17726473	46127994	*NCOA3*	C/T	0.062	0.0663	2.72E-08	0.068	0.0241	1.6E-01

The effect and alternative allele are designated A1 and A2, respectively. Effect size, standard error, p-value and number of specimens analysed are presented. SNP positions are relevant to human genome assembly GRCh37/hg19 The genome-wide significance threshold is p=5E-8, and the suggestive threshold is p=1E-5. Replication was significant where p<0.017 in the replication cohort.

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Table 4. SNPs in the meta-analysis reaching statistical significance on GWAS

Phenotype						Discovery			Replication			Meta-analysis		
	Chr	SNP	Position	Nearest gene(s)	A1/A2	Freq A1	Effect	P	Freq A1	Effect	P	Freq A1	Effect	P
TSH														
	6	rs11755845	43904780	*VEGFA/LINC01512*	T/C	0.237	-0.1799	3.31E-07	0.257	0.0434	4.43E-06	0.2557	-0.0525	7.70E-09
	9	rs1561961	100667599	*TRMO*	T/C	0.359	-0.1299	4.56E-05	0.37	-0.0434	4.29E-07	0.3693	-0.0492	2.44E-09
TPOAb														
	20	rs445219	46286239	*NCOA3/SULF2*	C/G	0.071	0.0611	5.24E-08	0.068	0.0444	9.10E-03	0.070	0.0561	2.12E-09
fT3														
	X	rs12687280	105307776	*SERPINA7*	T/C	0.131	-0.1552	1.54E-05	0.119	-0.2399	1.08E-07	0.1263	-0.1882	1.86E-11

The effect and alternative allele are designated A1 and A2, respectively. Effect size, standard error, p-value and number of specimens analysed are presented. SNP positions are relevant to human genome assembly GRCh37/hg19. The genome-wide significance threshold is *p*=5E-8, and the suggestive threshold is *p*=1E-5

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Table 5. Genes significantly associated with thyroid function on VEGAS2 analysis

Gene	Chr	Start	Stop	nSNPS	p value	Best SNP	SNP p-value	Phenotype
TRMO	9	100616770	100734852	162	1.0E-06	rs1561961	2.4E-09	TSH
FOXE1	9	100565536	100668997	203	1.0E-06	rs1561961	2.4E-09	TSH
HEMGN	9	100639072	100757197	124	1.0E-06	rs1561961	2.4E-09	TSH
NPAS3	14	33358458	34323382	638	1.0E-06	rs8003317	3.2E-06	fT4
SERPINA7	X	105227189	105332718	64	1.0E-06	rs12687280	1.9E-11	fT3

VEGAS2v2 gene-based analysis was limited to the coding area of the gene and 50kb upstream and downstream. Analysis window boundaries (designated by "Start" and "Stop") are relevant to human genome assembly GRCh37/hg19. Threshold for genome-wide significance is *p*=1.9E-6

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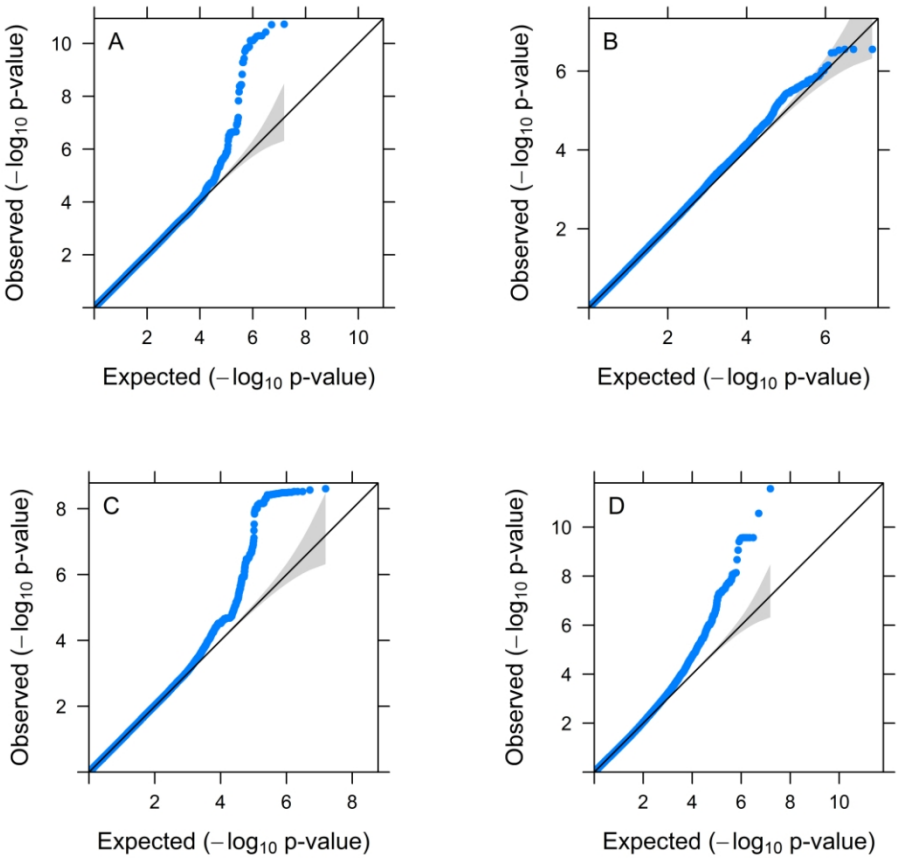

Supplemental Figure 1. QQ-Plots for SNP-based GWAS meta-analysis and associated lambda (λ) values (standard error) for each parameter. A) fT3: λ = 1.012 (7.55E-6), B) fT4: λ = 1.025 (3.43E-6), C) TSH: λ = 1.018 (1.19E-5), D) TPOAb: λ = 1.018 (1.62E-5).

347x322mm (87 x 87 DPI)

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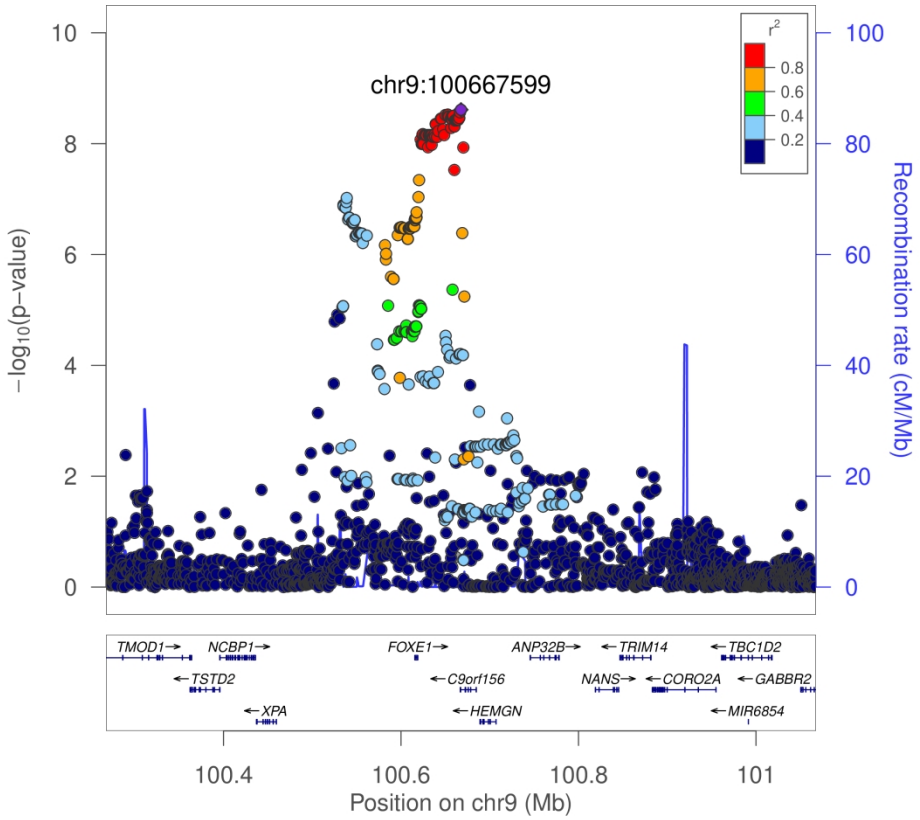

Supplemental Figure 2. Regional association plot for meta-analysis results. A) TSH at the 9q22.33 locus and B) TSH at the 6p21.1 locus. Genetic variants within 400kb of the lead variant are plotted by location on the x axis, with -log10 p value on the y axis. Colour fill of each data point reflects the LD (r2) with the lead variant (1000GP Nov 2014 EUR population). The position of genes in the locus (hg19) and recombination rate (blue line) is also indicated.

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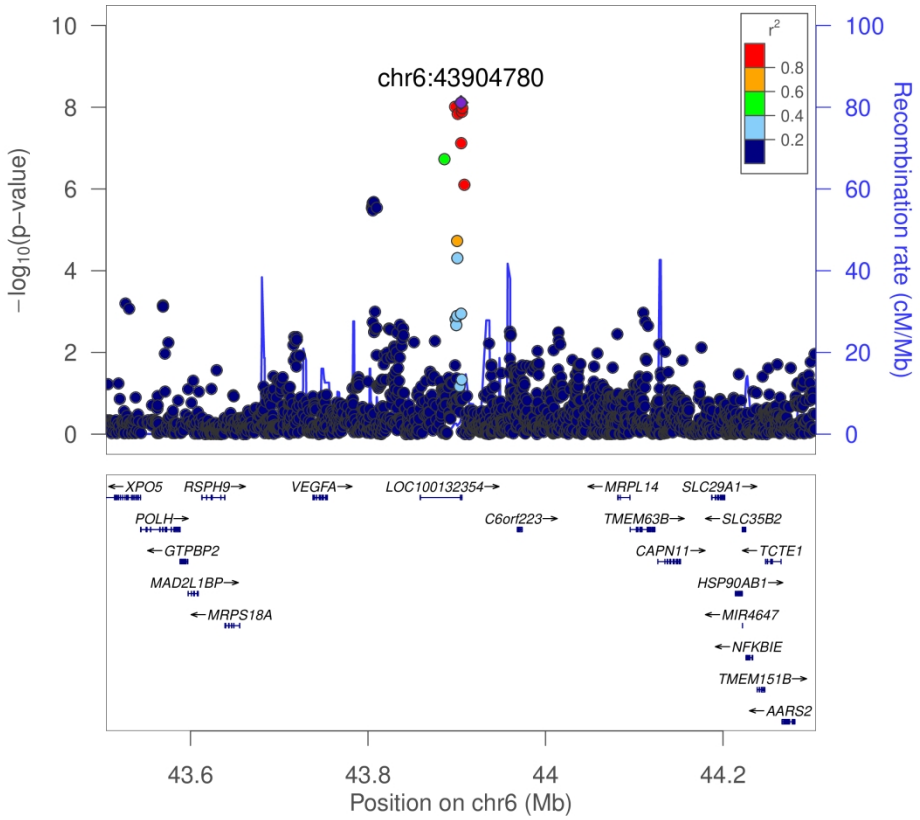

As per Supplemental Figure 2A. (multipanel figure)

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Supplemental Table 1. Intraclass correlation coefficients for covariate-adjusted thyroid function analytes

Age (years)		MZ twins	n	DZ twins	n
12	fT3	0.59 (0.51, 0.66)	277	0.29 (0.20, 0.37)	488
	fT4	0.69 (0.63, 0.75)	277	0.37 (0.29, 0.44)	488
	TSH	0.70 (0.64, 0.76)	277	0.47 (0.39, 0.53)	488
	TPOAb	0.38 (0.27, 0.47)	280	0.16 (0.07, 0.25)	491
14	fT3	0.63 (0.55, 0.70)	243	0.28 (0.20, 0.36)	470
	fT4	0.66 (0.59, 0.73)	243	0.30 (0.22, 0.38)	470
	TSH	0.73 (0.67, 0.79)	243	0.40 (0.32, 0.47)	470
	TPOAb	0.46 (0.36, 0.55)	246	0.31 (0.23, 0.39)	476
16	fT3	0.52 (0.40, 0.62)	163	0.23 (0.11, 0.34)	277
	fT4	0.70 (0.61, 0.77)	163	0.34 (0.24, 0.44)	277
	TSH	0.72 (0.64, 0.79)	163	0.15 (0.04, 0.27)	277
	TPOAb	0.55 (0.44, 0.65)	165	0.13 (0.02, 0.25)	281
Last	fT3	0.59 (0.54, 0.63)	683	0.27 (0.22, 0.32)	1235
	fT4	0.68 (0.64, 0.72)	683	0.34 (0.29, 0.39)	1235
	TSH	0.72 (0.68, 0.75)	683	0.37 (0.32, 0.42)	1235
	TPOAb	0.45 (0.39, 0.51)	691	0.21 (0.16, 0.26)	1248

Intraclass correlation coefficients (r) are shown with corresponding 95% confidence intervals and number of individuals sampled (n). All r were significantly different from zero at the $\alpha'=0.006$ significance threshold. Each difference in within twin pair correlation between zygosity groups ($r_{MZ} - r_{DZ}$) was statistically significant at the $\alpha'=0.013$ threshold. In all cases $r_{MZ} > r_{DZ}$ ($p < 0.013$). Last: using only the samples taken at a subject's final visit.

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