A Locus on Chromosome 1p36 Is Associated with Thyrotropin and Thyroid Function as Identified by Genome-wide Association Study

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Thyroid hormones are key regulators of cellular growth, development, and metabolism, and thyroid disorders are a common cause of ill health in the community. Circulating concentrations of thyrotropin (TSH), thyroxine (T4) and triiodothyronine (T3) have a strong heritable component and are thought to be under polygenic control, but the genes responsible are mostly unknown. In order to identify genetic loci associated with these metabolic phenotypes, we performed a genome-wide association study of 2,120,505 SNPs in 2014 female twins from the TwinsUK study and found a significant association between rs10917469 on chromosome 1p36.13 and serum TSH ($p = 3.2 \times 10^{-8}$). The association of rs10917469 with serum TSH was replicated ($p = 2.0 \times 10^{-4}$) in an independent community-based sample of 1154 participants in the Busselton Health Study. This SNP is located near *CAPZB*, which might be a regulator of TSH secretion and thus of pituitary-thyroid axis function. Twenty-nine percent of white individuals carry the variant, and the difference in mean TSH concentrations between wild-type individuals and those homozygous for the minor G allele was 0.5 mU/l, which is likely to be clinically relevant. We also provide evidence of suggestive association ($p < 5.0 \times 10^{-6}$) of other SNPs with serum TSH, free T4, and free T3 concentrations, and these SNPs might be good targets for further studies. These results advance understanding of the genetic basis of pituitary-thyroid axis function and metabolic regulation.

Acting on almost every tissue in the body, thyroid hormones affect basal metabolic rate, protein synthesis, fat and carbohydrate metabolism, and cellular response to catecholamines. They are essential to development and cellular differentiation in a range of tissues, as demonstrated by the severe clinical consequences of untreated congenital hypothyroidism [MIM #218700]. Clinically, one evaluates pituitary-thyroid axis function by measuring circulating concentrations of thyrotropin (TSH), free thyroxine (T4), and free triiodothyronine (T3). TSH and T4 have an inverse, log-linear relationship such that small changes in free T4 levels result in large changes in TSH secretion, making TSH the most sensitive marker of thyroid dysfunction.[1] In healthy subjects, TSH, free T4, and free T3 concentrations show considerable differences between individuals (inter-individual variation), whereas variability is much less in the same individual sampled repeatedly over a prolonged period of time (intra-individual variation).[2] This suggests that individuals have different set points (individual means) for pituitary-thyroid axis function. Twin and family studies have shown that these set points are under strong genetic influence; heritability estimates are up to 65% for variation in serum TSH, free T4, and free T3.[3–5] This could be clinically important because even in euthyroid subjects, small differences in thyroid function are associated with clinical parameters such as body mass index,[6–8] blood pressure,[9] lipids,[10] presence of atrial fibrillation,[11] and cardiovascular mortality.[12] Thyroid disease is an important public health problem in that it affects up to 10% of the population.

At present, little is known about which genes regulate pituitary-thyroid axis set points, and given the complexity of hypothalamo-pituitary-thyroid physiology, a large number of candidate genes exist. Thus far, only two single-nucleotide polymorphisms (SNPs) have been shown with convincing significance and rigorous replication to be associated with differences in circulating concentrations of TSH or thyroid hormones: one SNP is in the phosphodiesterase 8B gene *PDE8B* (MIM +603390) (associated with TSH),[13] and one is in the iodothyronine deiodinase 1 gene *DIO1* (MIM +147892) (associated with the T4/T3 ratio).[14] These SNPs account for only a small proportion of phenotypic variance, and it seems likely that other regulatory genes remain to be identified for TSH, free T4, and free T3.

To investigate this further, we undertook a genome-wide association study (GWAS) to attempt to identify genetic loci that influence serum TSH, free T4, and free T3 concentrations in healthy twins, and we replicated the key result in an independent cohort.

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The discovery cohort consisted of 2,456 female twins of European descent (2035 dizygotic and 421 monozygotic), aged 18 to 80 years, from St Thomas' UK Adult Twin Registry (TwinsUK), a volunteer sample recruited through a national media campaign in the United Kingdom without selection for particular diseases or traits.[15] Zygosity was determined by a standard questionnaire and by multiplex DNA fingerprinting with variable tandem repeats as well as concordance of genome-wide genotyping. Serum TSH, free T4, free T3, and thyroid peroxidase antibodies (TPOAb) were measured by chemiluminescence immunoassay with the Abbott Architect analyzer (Abbott Diagnostics, North Ryde, Australia). After exclusion of participants with a history of thyroid disease, those taking medications likely to influence serum thyroid function (such as anticonvulsants or oral glucocorticoids), and those with biochemical evidence of clinically significant thyroid dysfunction (defined as serum TSH < 0.1 or > 10 mU/liter), the study cohort consisted of 2364 participants. Genotyping was carried out via the Infinium assay (Illumina, San Diego, USA) with a combination of fully compatible SNP arrays (Hap300, Hap550 and Hap610). We pooled together the normalized intensity data for genomic DNA samples and used the Illluminus genotype-calling algorithm to assign genotypes. No calls were assigned if an individual's most likely call had a posterior probability less than 0.95. Validation of pooling was achieved via a visual inspection of 100 random, shared SNPs for overt batch effects; none were observed. We excluded subjects on the basis of the genotype data when (1) the SNP call rate was $< 90\%$ and (2) when there was evidence of non-European ancestry as determined by application of the STRUCTURE program.[16] We imputed ~2.5 million HapMap phase II (Build 36/hg18) SNPs for each participant by using IMPUTE software and used the "proper info" output variable to determine imputation quality. Only SNPs with an imputation quality value ≥ 0.4 were retained in the analysis. We employed additional quality criteria to filter imputed SNPs: $p < 1 \times 10^{-6}$ in a test for deviation from Hardy-Weinberg equilibrium (1,169 SNPs), minor allele frequency (MAF) $< 1\%$ (143,873 SNPs), and SNP call rate $< 90\%$ (295,759 SNPs). We thus retained 2,120,505 SNPs for analysis. The final study cohort with complete SNP data and thyroid function consisted of 2014 individuals.

The replication cohort consisted of 1154 participants in the 1994-5 Busselton Health Study. This is a cross-sectional health survey of inhabitants of a rural town in Western Australia with a predominantly white, iodine-sufficient population; details of the cohort have been published previously.[17] Serum TSH, free T4, free T3, and TPOAb were measured with an Immulite 2000 chemiluminescent analyzer (Diagnostic Products Corporation, Los Angeles, California). The exclusion criteria above were applied, and the result was a disease-free cohort of 1093 subjects. We attempted replication of eight SNPs selected from the GWAS results on the basis of the observed level of statistical significance and biological plausibility of genes in the locus. These SNPs were as follows: for TSH, rs10917469, rs13383344, rs1527680, and rs6030171; for free T4, rs211811, rs1908679, and rs7285004; and for free T3, rs10493251. The genotype for each SNP was determined via TaqMan allelic discrimination 5′ nuclease assays (Applied Biosystems, Foster City, CA) in a reaction volume of 5 μl containing assay-specific primers and allele-specific probes according to the manufacturer's protocol. Fluorescence was measured by a Victor2 Multilabel Plate-Reader (Perkin-Elmer, Waltham, MA). Informed consent was obtained from each individual, and the study was approved by the local ethics committee.

Table 1. Characteristics of the Two Cohorts

	Twins UK	Busselton (Replication Cohort)
Number	2,014	1,154
Female (percent)	100	51.9
Age (years)	46.4 (12.4)	59.4 (14.3)
BMI (kg/m^2)	25.2 (4.7)	26.4 (4.1)
TSH (mU/liter)	1.5 (0.8)	1.8 (2.1)
Free T4 (pmol/liter)	13.7 (1.7)	16.6 (3.4)
Free T3 (pmol/liter)	3.9 (0.6)	4.0 (0.9)
Percent TPOAb +ve	16.9%	16.5%

Mean (SD) is given unless otherwise stated.

Association testing of the thyroid phenotypes TSH, FT4, and FT3 was conducted with the GenABEL[18] suite of programmes. We applied the inverse normal transform to all phenotypes to achieve a normal distribution. Prior to analysis, phenotypes were adjusted for factors—age, sex (for the replication cohort only), positive TPO antibody status, and body mass index (BMI) —known to be associated with thyroid function. For the TwinsUK group, we used the quantitative-traits score test based on the method of Chen and Abecasis[19] and implemented in GenABEL, which accounts for the relatedness between individuals from the same family. In the replication cohort, the selected SNPs were analyzed with the score test of Aulchenko et al.[20] Descriptive statistics for both study groups were given as mean and standard deviation for continuous variables or percentages for categorical variables. All statistical analysis was performed in the R statistical computing environment, version 2.10.1.[21]

The clinical and demographic characteristics of participants in each cohort are shown in Table 1. Figure 1 shows the results of the GWAS by chromosome for TSH, free T4, and free T3. The strongest association with TSH was for rs10917469 at chromosome 1p36.13, and this result was significant ($p = 3.2 \times 10^{-8}$; Figure 2) at a genome-wide significance threshold of $p < 5.0 \times 10^{-8}$. This SNP is located in a linkage disequilibrium (LD) block extending ~47 kb. Numerous other SNPs that we genotyped in that

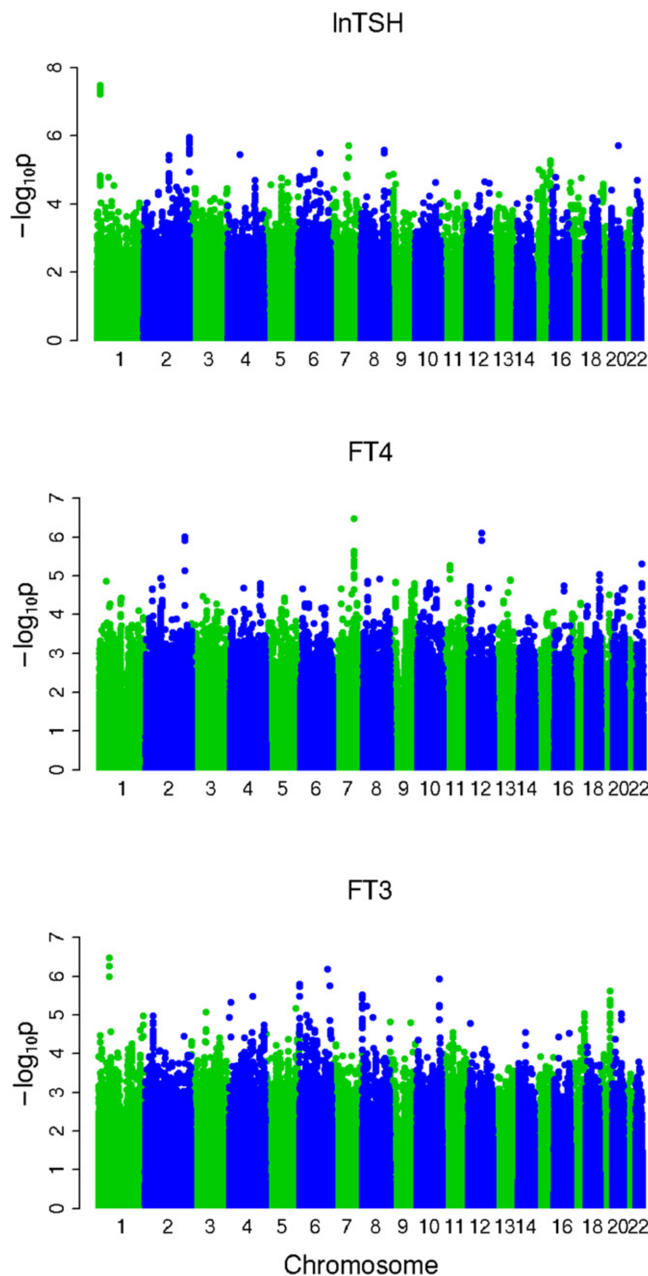

Figure 1. Manhattan Plots from GWAS of Serum TSH, Free T4, and Free T3 in the Discovery Cohort
A value of $p < 5.0 \times 10^{-8}$ is regarded as significant. For each marker, the −log10 of the p value resulting from an association test that evaluates its additive effect on the phenotype is plotted.

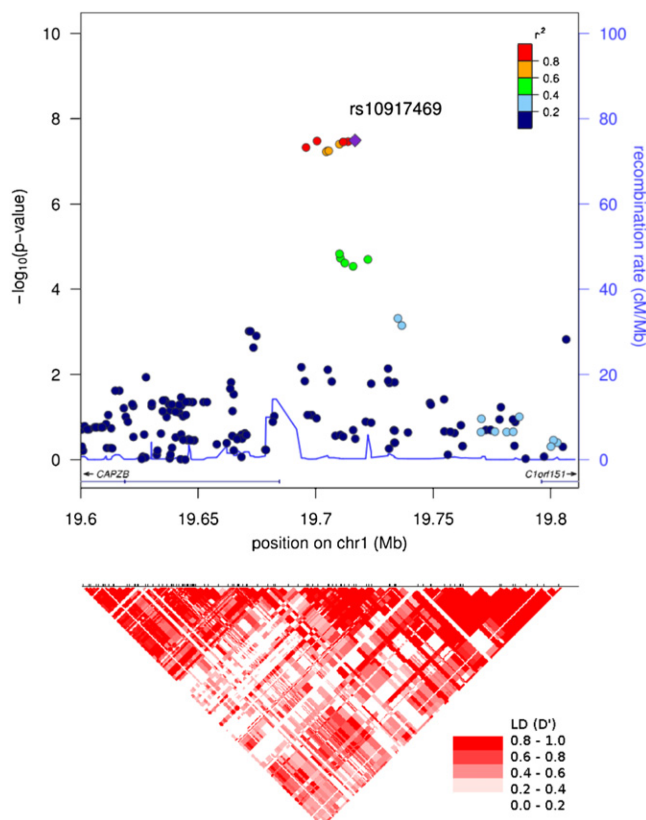

Figure 2. Association of SNPs at Chromosome 1p36.13 with Serum TSH in the Discovery Cohort and LD Structure, *D′*, of the Region on the Basis of HapMap CEU Data

genomic location showed a strong association with serum TSH levels in the GWAS (Figure 2), and this LD block is located approximately 10 kb upstream of the transcription start site of *CAPZB* (MIM *601572). *NBL1* (MIM *600613) and *C1orf151* are also located in this genomic region. Each G allele of rs10917469 was associated with a decrease in serum TSH concentrations. For free T4, the strongest association was with rs211811 on chromosome 7q31.1 ($p = 3.3 \times 10^{-7}$); the nearest gene is *IMMP2L* (MIM *605977), a mitochondrial inner-membrane peptidase located approximately 220 kb downstream. The SNP most strongly associated with serum-free T3 was rs10493251 ($p = 5.8 \times 10^{-7}$), which is in the vicinity of *DAB1* (MIM *603448) at chromosome 1p32.2. None of these SNPs are in genetic loci that were previously known to be associated with thyroid hormone levels. Associations with suggestive significance of $p < 5.0 \times 10^{-6}$ for each parameter are shown in Table S1. QQ plots of serum TSH, free T4, and free T3 are displayed in Figures S1, S2, and S3, respectively.

Of the provisional genetic associations with thyroid phenotypes that we selected for attempted replication in the Busselton cohort (Table S2), only one was confirmed: SNP rs10917469, located on chromosome 1p36.13 and associated with serum TSH. Table 2 shows that the association between rs10917469 genotype and serum TSH concentrations was similar in each cohort. After adjustment for relevant covariates, the difference in mean serum TSH concentration between the wild-type AA genotype and the alternative homozygote GG genotype was 0.5 mU/liter in the TwinsUK cohort and 0.4 mU/liter in the Busselton Health Study. Serum free T4 and free T3 concentrations did not differ significantly between individuals grouped by genotype. The total variance in TSH that is explained by rs10917469 after for other relevant covariates (age, TPOAb status, BMI and where appropriate, gender) are accounted for was 1.3% in the TwinsUK cohort and 1.0% in the Busselton cohort.

Table 2. Association of rs10917469 with TSH in the Discovery and Replication Cohorts

Cohort	n	A/A	A/G	G/G	freq (G)	beta (SE)	p Value
Twins UK	2006	1.5 (0.9)	1.4 (0.8)	1.0 (0.5)	0.16	−0.16 (0.03)	3.2×10^{-8}
Busselton	1049	2.0 (1.2)	1.9 (1.2)	1.6 (0.8)	0.15	−0.23 (0.06)	2.0×10^{-4}

Data are adjusted for sex, age, BMI, and TPOAb positivity. Values for each genotype are the mean (standard deviation) of TSH in mU/liter.

In the TwinsUK cohort, analysis of rs4704397 in *PDE8B*, the SNP previously reported as being associated with TSH,[13] showed an association of $p = 2.6 \times 10^{-3}$ and a per-allele effect size of 0.048 mU/liter. In our data this SNP explained 0.4% of the variance in TSH. Analysis of rs2235544 in *DIO1,* the SNP previously reported as being associated with free T4,[14] showed an association of $p = 9.4 \times 10^{-4}$ and a per-allele effect size of 0.18 pmol/liter, which explained 0.5% of the variance. These effect sizes are smaller than those reported in the original studies; however, the p values are consistent with the size of the cohort and effect sizes commonly reported for SNPs associated with multifactorial traits. Allowing for differences in the characteristics and exclusion criteria applied to the original study cohorts and this one, replication of these associations in our GWAS is reassuring about the validity of our findings.

The SNP rs10917469 is located upstream of *CAPZB*, which is not known to be involved in thyroid function. *CAPZB* encodes the beta subunit of the barbed-end actin-binding protein, a member of the F-actin-capping protein family. These proteins bind to the fast-growing ("barbed") ends of the actin filament blocking the exchange of subunits and regulating growth. Gene expression data (Unigene Hs.432760) reveals high levels of expression in normal thyroid compared with other tissues in humans and moderate expression in the brain and pituitary, making it biologically plausible that the gene regulates pituitary-thyroid axis function, although precisely how it does this is not known. Possible cellular pathways affected include cell-cycle spindle assembly, cytoskeleton remodeling, and assembly of cytoplasmic microtubules. The follicular architecture of the thyroid gland is important in thyroid physiology and is commonly disturbed in thyroid disease.[22] Organization of thyroid epithelial cells into structures enclosing colloid-filled lumena facilitates the synthesis and secretion of thyroid hormones and allows for this production to be coupled to a store of hormonal precursors.[22] Morphogenetic events including the coordinated action of cellular adhesion, intracellular trafficking, and precise regulation of morphogenetic cell movements are needed to create functional follicles. Furthermore, TSH acting through the cAMP-protein kinase A signaling pathway has been identified as a principal determinant of thyroid follicular morphology in vitro.[22] Therefore, a potential mechanism could involve sensitivity of thyrocytes to TSH and a response to cues embodied by cell shape, cytoskeletal tension, and actin nucleation and branching experienced in the adult context; this requires further study. The SNP rs10917469 is also located in the region of *NBL1*. The protein product of *NBL1* is reported to act as an antagonist for bone morphogenetic proteins by binding to them and preventing interaction with their receptors; therefore, the gene might play a role in growth and development.

SNP rs10917469 on chromosome 1p36.13 is only the second SNP—the other being rs4704397 in *PDE8B*[13]—convincingly shown to be associated with serum TSH concentrations. Each SNP accounts for only about 1% of population variance in serum TSH; this most likely reflects a polygenic influence on hormone variation. Other genes with small effects are likely to be discovered as larger study populations are used for GWAS. Despite this apparently modest effect, the difference of 0.5 mU/liter in mean serum TSH between wild-type and an alternative homozygote genotype for rs10917469 is likely to be clinically relevant. In population-based studies, small differences in serum TSH of the order of 0.5 to 1.0 mU/liter are associated with significant differences in blood pressure,[9] cholesterol,[10] BMI,[6–8] risk of atrial fibrillation[23] and cardiovascular mortality.[12] Furthermore, a comparable difference of 0.4 mU/liter between genotypes of the rs4704397 SNP in *PDE8B* has been shown to impact the diagnosis of subclinical hypothyroidism in pregnant women.[24] Further research is required to establish the contribution of these SNPs to clinically relevant health outcomes.

Strengths of our study include its large and well-characterized discovery cohort, detailed assessment (including measurements of free T4, free T3, and TPOAb) of pituitary-thyroid thyroid axis function, exclusion of participants with clinically relevant thyroid disease, and replication of the key finding in a separate, well-characterized cohort. A limitation of the study is that the replication cohort was of modest size, potentially reducing its power to replicate weaker associations from the discovery cohort. Using the Genetic Power Calculator software,[25] we estimated that in the replication study at an $\alpha = 0.01$, for variants explaining 1% of the trait variance and where the SNP is the quantitative trait nucleotide, the power is 0.80. However, if the proportion of the trait variance explained is lower, then the power of the replication study would be less. In addition, although we did attempt replication of what we considered the eight most biologically credible GWAS hits identified from the discovery cohort, this is a relatively small number of the SNPs. Study of a greater number of the top-ranked associations might have resulted in the confirmation of additional associations. Finally, although the SNP rs10917469 is close to *CAPZB*, which is expressed in human thyroid and pituitary tissue, it remains a possibility that the association observed with TSH is due to regulatory effects on another gene in this genomic region via linkage disequilibrium. Further

mapping studies of this locus and functional studies of *CAPZB* will be needed to clarify this detail.

In conclusion, we report the results of GWA scan for TSH, free T4, and free T3 in healthy individuals. We have identified a common variant that is close to *CAPZB* and is associated with serum TSH concentrations, and we replicated that finding in an independent cohort. We also report a number of other SNPs associated with these parameters in the discovery cohort, and these SNPs might warrant further study in other cohorts or meta-analyses of existing cohorts.

Supplemental Data

Supplemental Data include three figures and two tables and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), (http://www.ncbi.nlm.nih.gov/Omim)
IMPUTE software, (http://www.stats.ox.ac.uk/~marchini/software/gwas/impute)

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