ARTICLE

Phosphodiesterase 8B Gene Variants Are Associated with Serum TSH Levels and Thyroid Function

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Thyroid-stimulating hormone (TSH) controls thyroid growth and hormone secretion through binding to its G protein-coupled receptor (TSHR) and production of cyclic AMP (cAMP). Serum TSH is a sensitive indicator of thyroid function, and overt abnormalities in thyroid function lead to common endocrine disorders affecting ~10% of individuals over a life span. By genotyping 362,129 SNPs in 4,300 Sardinians, we identified a strong association ($p = 1.3 \times 10^{-11}$) between alleles of rs4704397 and circulating TSH levels; each additional copy of the minor A allele was associated with an increase of 0.13 μIU/ml in TSH. The single-nucleotide polymorphism (SNP) is located in intron 1 of *PDE8B*, encoding a high-affinity cAMP-specific phosphodiesterase. The association was replicated in 4,158 individuals, including additional Sardinians and two genetically distant cohorts from Tuscany and the Old Order Amish (overall p value $= 1.9 \times 10^{-20}$). In addition to association of TSH levels with SNPs in *PDE8B*, our genome scan provided evidence for association with *PDE10A* and several biologically interesting candidates in a focused analysis of 24 genes. In particular, we found evidence for association of TSH levels with SNPs in the *THRB* (rs1505287, $p = 7.3 \times 10^{-5}$), *GNAQ* (rs10512065, $p = 2.0 \times 10^{-4}$), *TG* (rs2252696, $p = 2.2 \times 10^{-3}$), *POU1F1* (rs1976324, $p = 3.9 \times 10^{-3}$), *PDE4D* (rs27178, $p = 8.3 \times 10^{-3}$), and *TSHR* (rs4903957, $p = 8.6 \times 10^{-3}$) loci. Overall, the results suggest a primary effect of *PDE8B* variants on cAMP levels in the thyroid. This would affect production of T4 and T3 and feedback to alter TSH release by the pituitary. *PDE8B* may thus provide a candidate target for the treatment of thyroid dysfunction.

Introduction

The thyroid controls several metabolic pathways through the synthesis and release of thyroxine (T4) and its active derivative triiodothyroxine (T3), which binds to nuclear receptors to regulate gene expression and impact growth, development, and metabolism.[1] Consistent with its critical role in muscle, bone, central nervous system, and heart physiology, abnormal thyroid function can lead to hyperthyroidism, hypothyroidism, and related childhood neuropsychological abnormalities including severe cretinism.[2]

The key regulator of thyroid function is thyroid-stimulating hormone (TSH). Secreted by the pituitary, TSH interacts with the TSH receptor (TSHR) on thyroid cells to upregulate cyclic AMP (cAMP) and induce Ca^{2+} release and activation of phosphoinositol.[3] These effectors then lead to the expression of downstream gene targets, culminating with pinocytosis of thyroglobulin and the release of T4 and T3, enhanced iodide uptake, and, on a longer time scale, growth and differentiation of thyroid follicular cells.

TSH levels are themselves controlled in several ways. TSH production is promoted by thyrotropin-releasing hormone (TRH) and inhibited by somatostatin, both produced by the hypothalamus.[4,5] In addition, negative-feedback control on hypothalamic release of TRH and pituitary release of TSH is exerted by the blood levels of thyroid hormone. When the levels of T3 and T4 are low, the production of TRH and TSH increases, and conversely, when T3 and T4 levels are high, TRH and TSH production decreases.[6]

Serum TSH concentrations are a sensitive indicator of thyroid function. High and low TSH levels reflect hypo- and hyperfunction of the thyroid gland, respectively. However, even within the normal range, TSH is a sensitive measure of thyroid function, and normal (euthyroid) individuals show narrow individual variation, suggesting that the thyroid-hormone axis is tightly regulated. TSH levels are genetically regulated and ~40% heritable in several populations; however, specific gene variants that influence TSH levels are not known.[7–9]

To identify specific possible genetic factors affecting TSH levels and thyroid function, we studied a Sardinian cohort[7] in whom the founder-population structure can simplify genetic analyses of complex traits and diseases. This has

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Table 1. Top SNPs Associated with TSH Levels from the GWA in SardiNIA

AffymetrixCode	Chr	Position	dbSNP	Allele (+/−)	Freq (+)	Effect[a]	SE[b]	H^2 [c]	p Value
SNP_A-2026717	**5**	**76554198**	**rs4704397**	**A/G**	**0.43**	**0.21**	**0.03**	**2.32%**	**1.3×10^{-11}**
SNP_A-2046779	**5**	**76566105**	**rs6885099**	**G/A**	**0.44**	**0.21**	**0.03**	**2.28%**	**1.8×10^{-11}**
SNP_A-1875128	**5**	**76571567**	**rs2046045**	**G/T**	**0.44**	**0.21**	**0.03**	**2.28%**	**3.0×10^{-11}**
SNP_A-4219180	9	133168819	rs657152	A/C	0.25	0.18	0.04	1.35%	2.4×10^{-7}
SNP_A-1799335	8	21883108	rs2291317	C/T	0.56	0.14	0.03	1.06%	5.5×10^{-6}
SNP_A-1860723	11	60939964	rs17704641	T/C	0.18	0.17	0.04	0.98%	5.6×10^{-6}
SNP_A-1952455	1	225872467	rs499689	A/G	0.57	0.14	0.03	1.04%	6.0×10^{-6}
SNP_A-4262573	2	222132815	rs2288629	A/C	0.77	0.16	0.04	0.96%	9.2×10^{-6}
SNP_A-1810205	8	21913213	rs17616085	G/A	0.56	0.14	0.03	1.00%	1.0×10^{-5}
SNP_A-2063584	8	21916371	rs3816786	T/A	0.56	0.14	0.03	0.99%	1.2×10^{-5}
SNP_A-1928725	2	85876014	rs4832006	T/C	0.18	0.17	0.04	0.97%	1.4×10^{-5}
SNP_A-2125590	**5**	**76524001**	**rs6453293**	**A/G**	**0.60**	**0.14**	**0.03**	**1.03%**	**1.9×10^{-5}**
SNP_A-1942513	8	21892330	rs1809498	C/G	0.55	0.13	0.03	0.94%	2.0×10^{-5}
SNP_A-1954621	3	184155898	rs7641401	T/C	0.71	0.14	0.03	0.90%	2.3×10^{-5}
SNP_A-4232593	8	21883779	rs4871903	A/G	0.54	0.13	0.03	0.97%	2.3×10^{-5}
SNP_A-2212237	2	17907614	rs300154	G/A	0.47	0.13	0.03	0.87%	2.6×10^{-5}
SNP_A-2148594	**5**	**76497909**	**rs4361497**	**G/C**	**0.69**	**0.14**	**0.03**	**0.88%**	**3.2×10^{-5}**
SNP_A-4265227	1	211014978	rs6656559	A/T	0.31	0.14	0.03	0.88%	3.3×10^{-5}
SNP_A-2071751	6	83147795	rs9344274	T/A	0.25	0.14	0.03	0.86%	3.4×10^{-5}
SNP_A-1849293	6	166027614	rs2983521	A/T	0.76	0.15	0.03	0.85%	3.6×10^{-5}
SNP_A-2293888	**5**	**76553738**	**rs13158164**	**C/G**	**0.11**	**0.20**	**0.05**	**0.83%**	**3.7×10^{-5}**
SNP_A-2039694	8	123942518	rs11779768	A/G	0.70	0.14	0.03	0.86%	3.8×10^{-5}
SNP_A-2276572	4	182013064	rs2545308	G/A	0.59	0.13	0.03	0.87%	3.8×10^{-5}
SNP_A-2192297	8	21889911	rs2306641	C/T	0.57	0.13	0.03	0.87%	3.9×10^{-5}
SNP_A-4274055	2	3406189	rs4849999	C/T	0.91	0.22	0.05	0.83%	3.9×10^{-5}

The table summarizes the top 25 association signals observed in the GWA scan in the Sardinian cohort. Chromosome assignments and physical position refer to the NCBI build 35 map. Alleles are ordered such that the first allele (+) is associated with increased TSH levels. SNPs in bold fall in the *PDE8B* gene region.

[a] The effect size is measured in standard-deviation units and is estimated as the β coefficient of the regression model when the normalized trait is used (e.g., an effect size of 1.0 implies that each additional copy of the allele being evaluated increases trait values by 1.0 standard deviation).

[b] SE represents the standard error of the effect.

[c] H^2 represents the amount of phenotypic variability explained by the marker and, thus, under an additive model, the amount of the heritability of the trait explained by the marker.

recently facilitated the finding of genes associated with susceptibility to asthma,[10] obesity-related traits,[11] uric acid,[12] lipid levels,[13] height,[14] and severity of β-thalassemia.[15] Here we report analyses that point to common variants in *PDE8B* [MIM 603390],[16] a gene that encodes a high-affinity cAMP-specific phosphodiesterase (PDE), as genetic modulators of TSH levels. The association was replicated in another group of Sardinians and in samples of Italians from Tuscany and of Old Order Amish from Pennsylvania.[17,18] Furthermore, our sample provides evidence for association with single-nucleotide polymorphisms (SNPs) in the *PDE10A* gene [MIM 610652],[19] as well as *thyroid-hormone receptor, β* (*THRB*) [MIM 190160][20] ($p = 7.3 \times 10^{-5}$) and *G protein, q polypeptide* (*GNAQ*) ($p = 2.0 \times 10^{-4}$)

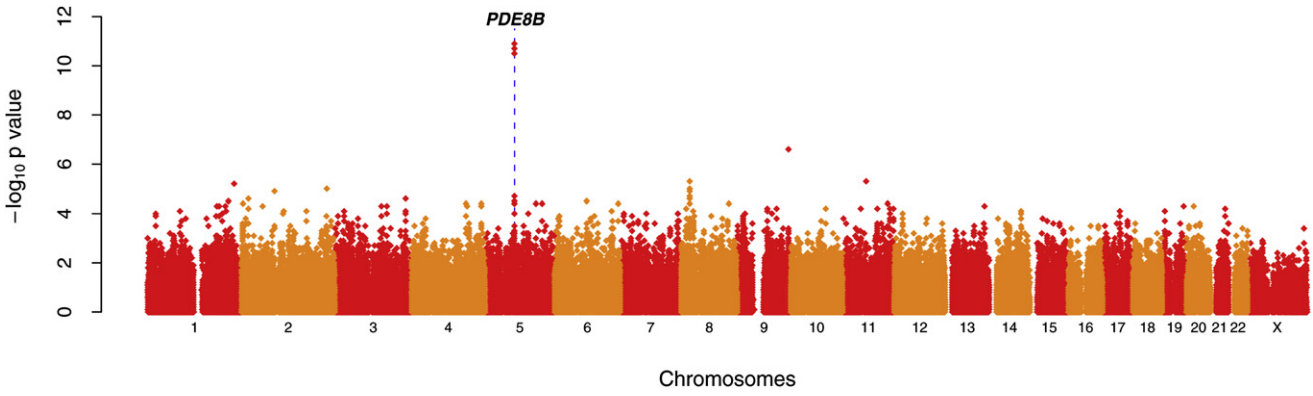

Figure 1. Results of Genome-wide Association Scan for TSH Levels

For each marker, the $-\log_{10}$ of the p value resulting from an association test that evaluates its additive effect on the phenotype is plotted. The position of *PDE8B* is annotated.

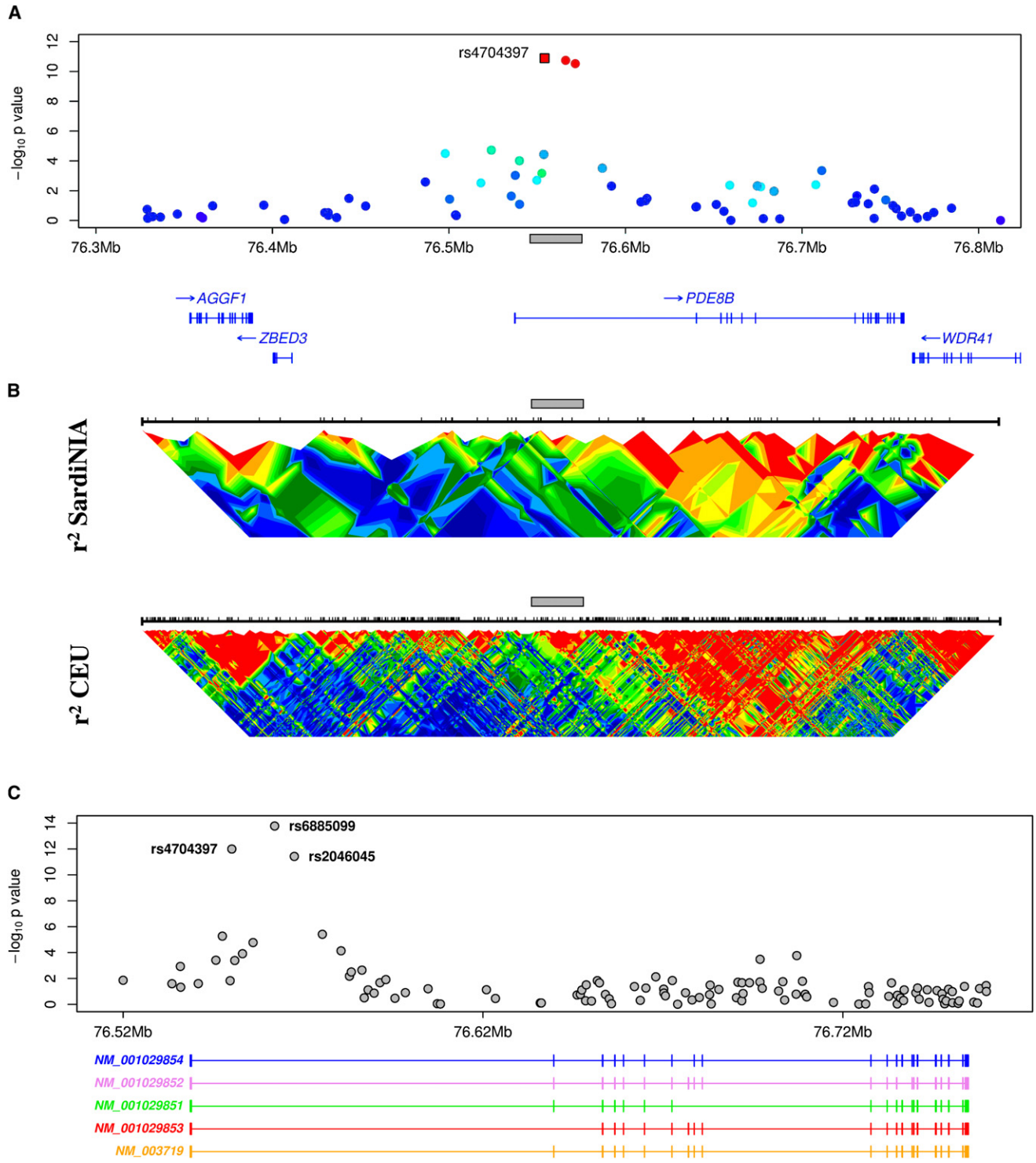

Figure 2. Association with TSH Levels and Linkage-Disequilibrium Patterns in the Region Surrounding *PDE8B*

(A) The top panel summarizes association between the SNPs and TSH levels in each individual ($-\log_{10}$ of the p value). The SNP showing strongest association (rs4704397) is highlighted and indicated with a red square. Other SNPs are colored according to their degree of disequilibrium with rs4704397, ranging from high (red) to intermediate (green) to low (blue). r^2 values of rs4704397 with rs6885099 and rs2046045 (red dots) are $r^2 = 0.98$ and $r^2 = 0.97$, respectively. The transcript for all genes in the region is indicated in the next panel, with an arrow indicating transcript direction.

(B) The two panels summarize the patterns of disequilibrium in SardiNIA and in the CEPH from Utah (CEU; Utah residents with ancestry from northern and western Europe) HapMap populations. r^2 values are colored as in (A) with GOLD,[30] which schematically draws the LD plot according to the physical position of markers, resulting in a line divided according to marker-marker distance. The gray bar marks the region of association and facilitates comparisons between the panels.

[MIM 600998],[21] among genes in a focused analysis of 24 candidates for involvement in the dynamic regulation of thyroid function. Overall, our results suggest that *PDE8B*, by affecting cAMP concentrations in the thyroid, may alter thyroid-hormone levels in the serum and affect TSH release from the pituitary. *PDE8B* variants thus modulate thyroid physiology and may affect the course of thyroid disease.

Material and Methods

Sample Description

We recruited and phenotyped 6,148 individuals, males and females, ages 14–102 yr, from a cluster of four towns in the Lanusei Valley of Sardinia.[7] During physical examination, a blood sample was collected from each individual and divided into two aliquots. One aliquot was used for DNA extraction and the other to characterize several blood phenotypes, including evaluation of serum TSH levels. TSH was measured with the Siemens TSH assay (Immulite 2000) according to the manufacturer's instruction. The method is a solid-phase, chemiluminescent, competitive analog immunoassay and has analytical sensitivity of 0.004 μIU/ml and upper limit of 75 μIU/ml of TSH. This method is a third-generation TSH assay and has a sensitivity and detection range comparable to the methods used to evaluate TSH levels in the other cohorts studied (see below).

Thyroid ultrasound examination was also performed on all the individuals enrolled in the study, with a portable real-time instrument using a 7.5-MHz linear transducer. Subjects were examined in the supine position, with neck hyperextended, by transverse and longitudinal scans to evaluate overall thyroid size and echotexture. Thyroid volume was calculated for each lobe according to the ellipsoid formula (length × breadth × width × 0.523) (normal range 10.7 ± 4.6 ml to 11.5 ± 3 ml). Goiter was scored when the total thyroid volume was above the mean thyroid volume. Reduction of thyroid volume associated with diffuse alteration of echotexture indicative of chronic thyropathies was also detected. Presence, structure, size, and vascularization of nodules were determined by ultrasound and color-Doppler sonography. Records of self-reported thyroid disease status (i.e., autoimmune thyroiditis, thyroid cancer, partial or total thyrectomy) and hormone-replacement therapy were available for all subjects.

Each participant signed an informed consent form. All study methods have been approved by the local ethics committee.

GWAS Genotyping

During the study, we genotyped 4,305 individuals selected from the whole sample to represent the largest available families, regardless of their phenotypic values. Specifically, 1,412 were genotyped with the 500K Affymetrix Mapping Array Set and 3,329 with the 10K Mapping Array Set, with 436 individuals genotyped with both arrays. This genotyping strategy allowed us to examine the majority of our cohort in a cost-effective manner because genotypes for the SNPs that passed quality-control checks could be propagated through the pedigree via imputation.[11,22] TSH measurements were available for 4,300 individuals among the 4,305 genotyped. A total of 362,129 SNPs passed initial quality-control checks[11] and were tested for association with TSH levels with an additive model. Further details of the strategy for imputation and data analysis are given in the Statistical Analysis section below. An additional 1,236 individuals were genotyped with the 10K Mapping Array Set but were not included in the genome-wide association scan (GWAS) because no close relatives were typed with the 500K Mapping Array Set. These individuals were, however, included in the fine-mapping analysis.

Replication and Fine Mapping

We designed a ParAllele Custom Chip (from Affymetrix) to replicate and refine the regions associated with TSH levels as well as other traits studied in the SardiNIA Project.[7,15] Specifically, for the locus associated with TSH levels, we included 70 markers that were chosen as SNP tags of markers falling in the coding regions of the *PDE8B* gene and that also passed reliability criteria in the design phase. SNP rs6885099 showed the strongest association with TSH levels in an initial analysis performed on a subset of phenotyped individuals and was thus included in the custom chip (instead of rs4704397, which ranked second in the initial analysis). Genotyping was performed in Sardinian individuals selected for replication or for fine-mapping efforts. In particular, for replication, we genotyped and analyzed 1,858 individuals from the SardiNIA cohort (stage 2), who were unrelated (kinship coefficient = 0) to the individuals in stage 1.

For fine mapping, we genotyped a subset of 634 individuals from stages 1 and 2 including 100 couples (mothers and fathers) already typed with the 500K Mapping Array Set and 434 individuals representing children from the larger sibships.

InCHIANTI

The InCHIANTI study is an ongoing epidemiological study in two Italian towns in the Chianti area (Tuscany). A detailed description of the study design and data-collection methods has been published.[17,23] Plasma TSH levels were measured with commercial kits (Vitros TSH Reagent, Ortho-Clinical Diagnostics, Johnson & Johnson Medical S.p.A Section) by chemiluminescent assay. The assay has an analytical sensitivity of 0.003 μIU/ml. The intra-assay coefficients of variation (CVs) were 3.9% to 5.3% over the range 0.06–80.11 μIU/ml. SNP rs4704397 was genotyped in this cohort by TaqMan Single SNP genotyping assays (Applied Biosystems).

Old Order Amish

The Old Order Amish (OOA) study participants reported here were from ongoing studies of cardiovascular disease and longevity; none were ascertained on the basis of thyroid disease.[18,24] A total of 1,136 individuals from these two studies had serum TSH measured by Quest Diagnostics (Nichols Institute) with a standardized third-generation assay and were previously genotyped for rs4704397 with the 500K Affymetrix Mapping Array Set.

Statistical Analysis

To evaluate the additive effect of each marker, we fitted a simple regression model and used a variance-component approach to account for correlation between different observed phenotypes within each family. Gender, age, and age squared were included

(C) The bottom panel summarizes the results of the fine mapping in the *PDE8B* gene. The association results are for all SNPs in the SardiNIA sample genotyped with either the 500K or 10K Mapping Array Set or the ParAllele custom chip. The top three markers, all in intron 1, are highlighted. The transcripts for all the *PDE8B* isoforms are indicated at the bottom.

as covariates in all analyses, and the trait was normalized with quantile normalization to avoid inflation of type I error rates. For individuals genotyped with a sparse map, we used a modified version of the Lander-Green algorithm[25,26] to estimate IBD sharing at the location of the SNPs being tested and identify stretches of haplotype shared with close relatives who were genotyped at higher density and probabilistically infer missing genotypes.[22] In brief, in the statistical approach to estimate each genotype, we first calculated the likelihood of the observed genotype data. Then, we assigned each missing genotype to a specific value and updated the likelihood pedigree. The ratio of the two likelihoods gave a posterior probability for the proposed genotype conditional on all available data. Furthermore, instead of assigning the most likely genotype, we estimated an expected genotype score, representing the expected number of copies of a reference allele (a fractional number between 0 and 2), which allowed us to partially account for uncertainty in genotype assignment. The genotype scores were then used in a variance-component-based association test as described.[22] Because of computational constraints, we divided large pedigrees into subunits with "bit-complexity"[26] of 19 or less (typically, 20–25 individuals) before analysis. False-discovery rates (FDRs) were calculated with R's p.adjust() procedure via the method of Benjamini and Hochberg.[27]

In SardiNIA stage 2 replication and in InCHIANTI samples, association with TSH was tested as in the SardiNIA GWA sample, except that no imputation was required. Association analyses to test for additive effects in the Old Order Amish sample were carried out under a variance-component framework implemented in SOLAR;[28] these analyses modeled the effect of genotype on TSH levels while adjusting for the effects of age and sex and accounting for a background polygenic contribution to variation in TSH levels occurring because of the relationships existing among the examined individuals.[29] The choice of SOLAR was based on the presence of larger pedigrees in the OOA families and the lack of a requirement for genotype imputation in these samples.

For fine mapping in the SardiNIA sample, we merged all available genotypes from all the platforms (500K Mapping Array Set, 10K Mapping Array Set, ParAllele custom chip) for a total of 6,062 nonoverlapping individuals. Because fewer markers were analyzed than in the GWAS, larger pedigrees were divided into units of bit-complexity of 21 or less before analysis. Missing genotypes for individuals typed with only one platform were inferred with the same approach used in the GWA analysis. We then evaluated the additive effect of each marker by using allele dosages as above.

Results

Genome-wide Association Scan for TSH Levels in Sardinians

Genome-wide association analyses were carried out in 4,300 volunteers characterized for circulating TSH levels (see Material and Methods).[7,11] Analyses revealed three SNPs with genome-wide significant association at a single chromosome 5 locus ($p < 10^{-10}$, see Table 1 and Figure 1).

These SNPs and three other SNPs among our top 25 signals lie in intron 1 or upstream of the *PDE8B* gene (Table 1 and Figure 2).[16] Their locations and linkage-disequilibrium (LD) relationships in the SardiNIA and CEU HapMap populations are illustrated in Figure 2.[30,31] A quantile-quantile

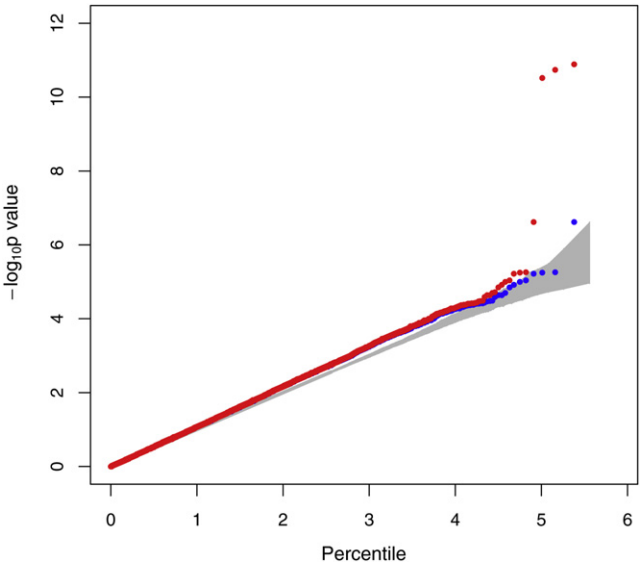

Figure 3. Quantile-Quantile Plot of SNPs Associated with TSH Level in the SardiNIA Study

Red dots represent all the 362,129 SNPs analyzed in the GWAS. Blue dots represent all analyzed SNPs not located within the *PDE8B* gene. The gray area corresponds to the 90% confidence region from a null distribution of p values generated from 100 simulations.

(QQ) plot (Figure 3) shows that most of the deviation from the null hypothesis is accounted for by markers falling in the *PDE8B* gene. After those markers were removed, the corresponding QQ plot (blue line) suggests that there are nevertheless likely to be some additional true signals (see below). The three SNPs that showed the strongest genome-wide significant association (rs4704397, $p = 1.3 \times 10^{-11}$; rs6885099, $p = 1.8 \times 10^{-11}$; rs2046045, $p = 3.0 \times 10^{-11}$) are all in strong linkage disequilibrium (Figure 2, average pairwise $r^2 > 0.98$). Among these, the SNP showing strongest association, rs4704397, explains 2.3% of the variance of TSH levels. Each copy of the minor A allele is associated with an average increase of 0.13 μIU/ml in TSH levels.

Replication of the Association of rs4704397/PDE8B in Other Populations

To confirm the association of rs4704397 with TSH levels, we analyzed two additional cohorts, one comprising 1,164 Tuscan subjects from the genetically distinct InCHIANTI study population[17,23] and the second 1,136 individuals from the founder population of the Old Order Amish.[18,24] We also analyzed a replication cohort of 1,858 individuals enrolled in the SardiNIA study but unrelated to the first group (designated here as stage 2 analysis). The features of these cohorts are given in Table 2, and the results of association are summarized in Table 3. Analysis revealed that SNP rs4704397 or its surrogate rs6885099 was associated with the TSH trait in all the cohorts tested, with the same direction of effect as observed in our original sample (Table 3). Notably, the effect size in the replication samples

Table 2. Characteristics of Samples Used in Genome-wide and Follow-Up Analyses

Population Study	n (% female)	Mean Age (SD)	Mean TSH (SD)	% Under Treatment	% Recorded Disease Status
SardiNIA	4300 (56.2%)	43.6 (17.6)	2.04 (3.2)	2.5%	81%
Follow-up samples					
SardiNIA stage 2	1858 (59.9%)	44.6 (17.4)	2.23 (4.4)	2.2%	81%
InCHIANTI	1164 (55.9%)	68.8 (15.4)	1.92 (4.7)	6.1%	100%
Old Order Amish	1136 (47.0%)	49.9(16.7)	2.19 (2.5)	6.8%	NA

The table shows the mean and standard deviation (SD) for age and TSH values for each study group, as well as the percentage of individuals under thyroid-hormone therapy and of individuals with clear reports of disease status (i.e., thyroid cancer, surgery, and autoimmune thyroiditis). NA, not assessed.

was about half that observed in the initial GWAS (0.25 versus 0.12). This may be due to less accurate estimates of effect size as a result of reduction in sample size and consequent decrease of statistical power, or it may reflect the "winner's curse" phenomenon, where effect-size estimates for markers that reach statistical significance may be inflated. The combined p value from stage 2 analyses in the three cohorts was highly significant ($p = 2.0 \times 10^{-10}$), leading to a cumulative-association p value of 1.9×10^{-20}.

Table 3 also summarizes the distribution of the TSH levels for each genotype class for the SNP rs4704397 and shows that in all the samples, individuals carrying at least one copy of the minor A allele have higher circulating levels of TSH than those homozygous for the major G allele, showing a trend for an additive model. In the Amish sample, the mean TSH value for individuals with the A/G genotype appears slightly higher than that observed for the A/A subjects. This could result from the different frequency of the rare A allele and the lower frequency of the A/A genotype in the Amish compared with the other cohorts; this lower frequency would yield less accurate mean-value estimates for this genotype class compared to values in the other cohorts.

We also repeated association analyses in all cohorts after stratifying subjects into two groups, one group including individuals with TSH levels in the normal range (0.4–4.0 μIU/ml), with no reported history of thyroid disease, and not taking thyroid medications (group 1, Table 4), and the other group including the remaining subjects (group 2). The results confirmed the association of rs4704397 in both the unaffected and the thyroid-affected individuals (Table 4). Because of the reduction in sample size, the p value was lower in the two subsets, but the effect size of the associated A allele was larger in group 2 individuals (in all but the OOA cohort), suggesting a potential involvement of *PDE8B* activity in the course of thyroid disease. Further analysis in a larger subgroup of thyroid-affected Sardinian subjects, including all the individuals from group 2 as well as individuals with nodules and goiters (formerly in group 1), showed stronger association when compared to the remaining subjects, again confirming a larger effect size of the A allele in thyroid-affected individuals (see Table S1 available online). Despite the consistently larger effect in thyroid-affected individuals in several studies, this difference does not reach statistical significance (Cochran's Q statistic[32] $p = 0.49$, $I^2 = 0\%$); consequently, larger studies will be necessary to test this hypothesis further. Association analysis of rs4704397 in males and females did not suggest gender-specific effects (Table S2). Signals for other loci that did not reach genome-wide significance (Table 1) did not replicate strongly in other cohorts and have in general not been followed up further. For two SNPs, rs657152 on chromosome 9 (in the *ABO blood group* gene [MIM 110300], with borderline Bonferroni p value) and SNP rs2983521 on chromosome 6 (in *PDE10A*,[19] a gene that belongs to the same enzyme family

Table 3. Summary of Association Results for SNP rs4704397 in All Cohorts

Population Study	n	A/A	A/G	G/G	Freq(A)	Effect[a] (SE)	p Value
SardiNIA[b]	4300	1.60 (1.50)	1.38 (1.21)	1.20 (1.14)	0.44	0.25 (0.04)	1.3×10^{-11}
Stage 2							
SardiNIA stage 2[c]	1858	1.84 (1.43)	1.67 (1.30)	1.49 (1.32)	0.44	0.13 (0.03)	1.1×10^{-4}
InCHIANTI	1164	1.50 (2.12)	1.32 (1.14)	1.26 (1.18)	0.41	0.12 (0.04)	0.0021
Old Order Amish	1136	1.75 (1.54)	1.80 (1.42)	1.55 (1.33)	0.33	0.12 (0.05)	7.0×10^{-5}
							Meta-analysis
Stage2	4158						2.0×10^{-10}
Overall	8458						1.9×10^{-20}

The table summarizes association results for rs4704397 in all cohorts. For each study, genotype medians (IRQ range) of unadjusted TSH values are reported in μIU/ml; effect sizes (SE) and p values are given relative to the normalized trait.

[a] The effect size is measured in standard-deviation units and is estimated as the β coefficient of the regression model when the normalized trait is used (e.g., an effect size of 1.0 implies that each additional copy of the allele being evaluated increases trait values by 1.0 standard deviation).

[b] SardiNIA genotype medians are relative only to individuals directly genotyped with the 500K Mapping Array Set.

[c] Results are for rs6885099, in strong LD ($r^2 = 0.98$) with rs4704397, which was not genotyped (see Material and Methods).

Table 4. Results of Association Analysis of rs4704397 in Healthy and Thyroid-Affected Individuals

	Group 1			Group 2		
Population Study	**n**	**Effect[a] (SE)**	**p Value**	**n**	**Effect[a] (SE)**	**p Value**
SardiNIA	3785	0.18 (0.03)	1.5×10^{-8}	515	0.26 (0.08)	1.3×10^{-3}
Stage 2						
SardiNIA stage 2[b]	1645	0.12 (0.03)	7.9×10^{-4}	213	0.24 (0.10)	0.013
InCHIANTI[c]	994	0.08 (0.05)	0.067	191	0.24 (0.10)	0.016
Old Order Amish[d]	962	0.15 (0.05)	5.7×10^{-4}	174	0.11 (0.11)	0.104
			Meta-analysis			**Meta-analysis**
Stage 2	3601		5.4×10^{-7}	578		1.5×10^{-4}
Overall	7386		4.3×10^{-14}	1093		7.2×10^{-7}

We compared association results in two subgroups. Group 1 identifies healthy subjects and consists of individuals with TSH levels in the normal range of 0.4–4 μIU/dl and with no history of thyroid disease (thyroid medications, thyroid cancer, surgery, or autoimmune thyroiditis). Group 2 consists of the remaining subjects.

[a] The effect size refers to the minor allele frequency (MAF) allele; it is measured in standard-deviation units and is estimated as the β coefficient of the regression model when the normalized trait is used (e.g., an effect size of 1.0 implies that each additional copy of the allele increases trait values by 1.0 standard deviation).

[b] Results are for rs6885099, in strong LD ($r^2 = 0.98$) with rs4704397, which was not genotyped (see Materials and Methods).

[c] Thryoid autoimmunity and thyroid cancer information were not reported in the InCHIANTI database. Old Older Amish database did not contain any information regarding thyroid disease, so only out-of-range TSH values and history of taking thyroid-hormone therapy were considered to define group 2.

[d] Old Older Amish database did not contain information regarding thyroid disease, so only out-of-range TSH values and history of taking thyroid-hormone therapy were considered to define group 2.

as *PDE8B*), some further testing was performed. SNP rs657152 did not replicate in the Amish or InCHIANTI cohorts, whereas rs2983521 was replicated in InCHIANTI, with a one-sided p value of 0.048, but was not supported in the Amish samples (data not shown). Thus, its involvement in the variability of TSH remains suggestive, requiring study in additional cohorts.

Fine Mapping in the *PDE8B* Locus

To identify possible coding and/or functional variants involved in the regulation of *PDE8B* activity in the context of TSH variation, we genotyped 70 additional SNPs falling in the *PDE8B* gene or in the neighboring 5 kb upstream and downstream regions in 2,492 Sardinians. We analyzed all the available markers (39 from the GWAS and 70 from the custom chip) in the individuals genotyped with at least one platform and for whom TSH values were available (n = 6,062). As shown in Figure 2C and Table S3, the strongest association signals all pointed to intron 1, common to all five *PDE8B* isoforms, in which ten significant SNPs were located. The top three SNPs confirmed the previous top three markers detected in the GWAS, though with a shifted rank order (rs6885099, $p = 1.69 \times 10^{-14}$; rs4704397, $p = 1.01 \times 10^{-12}$; rs2046045, $p = 3.76 \times 10^{-12}$). Several of the SNPs show linkage disequilibrium ($r^2 > 0.5$) with SNPs in neighboring regions (Figures 2A and 2B), suggesting that the functional variant(s) may lie outside of intron 1. However, it seems likely that they will reside in a noncoding sequence, because sequence analysis of all the *PDE8B* exons in 20 subjects homozygous for the rare and 20 for the common alleles of the top two associated SNPs, rs4704397 and rs6885099, did not reveal any coding variants (data not shown). In addition to intron 1, we also found evidence of association for two new SNPs within intron 10 of *PDE8B1*. The functional relevance of these variants remains to be elucidated. Further studies, including large-scale resequencing and molecular analyses, will be necessary to verify the effects of the associated variants on the regulation of the TSH levels.

Association Analysis of Candidate Genes with TSH Levels

Our GWA analysis implicated *PDE8B* as the only gene with a significant effect on variation of TSH. Because *PDE8B* variants contribute only 2.3% of TSH variation, which represents a small part of the total genetic component of the trait (~40%), we expected other genes to contribute to TSH levels, albeit with smaller effects. To begin to look for such genes, we carried out in the SardiNIA cohort a subanalysis focusing on candidate genes, where a less stringent significance threshold would increase power. We selected 24 candidate genes on the basis of their reported function in cAMP metabolism, TSH signaling, and the regulation of thyroid function. No linkage and association studies showing a correlation with TSH levels had previously been performed for any of these candidates. When markers falling in the candidates and already genotyped in the GWAS were tested for their association with TSH levels, as expected none reached genome-wide significant association (Table 5). However, substantive evidence of association was detected for some genes involved in the biological activity and negative feedback of thyroid hormone (*THRB*),[20] in signaling of TSH (*TSHR* [MIM 603372] and *GNAQ*),[21,33] in thyroid-hormone production (*TG* [MIM 188450]),[33] and in phosphodiesterases involved in the catabolism of cAMP (*PDE4D* [MIM 600129])[34,35] (see Discussion for more details). Among the various PDE genes, *PDE4D* and *PDE7B* [MIM 604645][36] were included in the

Table 5. Tag SNPs that Show Strongest Association with TSH in Previously Identified Candidate Genes in the TSH Signaling Pathways and Thyroid Function

Gene	# Affymetrix SNPs	# HapMap SNPs	Coverage (0.5)	Coverage (0.8)	Best p Value	SNP	Allele (+/−)	Freq (+)	Effect[a]	H2
THRB	**58**	**382**	**0.67**	**0.45**	**7.3×10^{-5}**	**rs1505287**	**G/A**	**0.67**	**0.16**	**1.27%**
GNAQ	**20**	**204**	**0.91**	**0.75**	**2.0×10^{-4}**	**rs10512065**	**G/A**	**0.85**	**0.13**	**0.49%**
TG	**76**	**489**	**0.95**	**0.86**	**2.2×10^{-3}**	**rs2252696**	**A/C**	**0.47**	**0.08**	**0.37%**
POU1F1	**1**	**9**	**1.00**	**0.78**	**3.9×10^{-3}**	**rs1976324**	**G/A**	**0.66**	**0.12**	**0.74%**
PDE4D	**119**	**714**	**0.82**	**0.61**	**8.3×10^{-3}**	**rs27178**	**G/A**	**0.46**	**0.07**	**0.24%**
TSHR	**21**	**221**	**0.87**	**0.61**	**8.6×10^{-3}**	**rs4903957**	**G/A**	**0.69**	**0.09**	**0.34%**
GNAO1	**22**	**157**	**0.87**	**0.70**	**0.01**	**rs2550298**	**T/C**	**0.52**	**0.05**	**0.11%**
DIO1	**5**	**16**	**0.88**	**0.69**	**0.03**	**rs2294512**	**G/A**	**0.83**	**0.12**	**0.43%**
PDE7B	**39**	**196**	**0.85**	**0.61**	**0.03**	**rs9376165**	**G/A**	**0.82**	**0.14**	**0.60%**
GNAI1	**8**	**70**	**0.83**	**0.40**	**0.04**	**rs2523189**	**C/T**	**0.76**	**0.10**	**0.32%**
GNAS	**6**	**36**	**0.61**	**0.39**	**0.05**	**rs6026565**	**A/T**	**0.14**	**0.06**	**0.09%**
TTF1	0	19	0.32	0.16	0.08	rs7030233	G/A	0.18	0.08	0.23%
TPO	17	176	0.81	0.53	0.09	rs2071403	G/A	0.54	0.09	0.45%
DIO2	0	12	0.83	0.67	0.09	rs9919906	C/T	0.59	0.08	0.32%
TSH-A	2	11	1.00	0.91	0.13	rs6155	C/T	0.06	0.09	0.11%
PAX8	10	41	0.88	0.66	0.16	rs6716573	T/C	0.22	0.08	0.24%
PROP1	0	3	0.67	0.00	0.23	rs4413548	T/C	0.51	0.02	0.03%
TRHR	8	37	0.86	0.70	0.25	rs3134115	T/C	0.44	0.02	0.02%
CREBBP	8	23	0.61	0.57	0.29	rs8046065	C/T	0.81	0.01	0.00%
CREB1	2	19	0.74	0.74	0.32	rs2709393	A/G	0.06	0.02	0.00%
CTLA4	0	2	1.00	0.50	0.36	rs11571292	G/A	0.80	0.03	0.03%
TSH-B	0	4	1.00	1.00	0.66	rs2274118	G/C	0.28	0.02	0.02%
THRA	1	4	0.25	0.25	0.84	rs939348	C/T	0.85	0.00	0.00%
PRKAR1B	0	2	0.00	0.00	NA	NA	NA	NA	NA	NA

The first column indicates the name of a previously identified candidate gene. The second column indicates the number of SNPs in our Affymetrix arrays that are within ± 5 kb of the gene. The next column indicates the number of HapMap SNPs within ± 5 kb of the gene and the proportion of these that are covered at $r^2 = 0.50$ or $r^2 = 0.80$ by SNPs in the Affymetrix arrays. The remaining columns indicate the SNP that showed strongest association in our analysis, the p value, the tested allele and its frequency, and the estimated additive effect in standard-deviation units. Association was tested either with intragenic SNPs or with the best available tag for intragenic SNPs ($r^2 > 0.5$). Shown in bold are candidate genes with SNPs reaching p values ≤ 0.05.

[a] The effect size refers to the MAF allele; it is measured in standard-deviation units and is estimated as the β coefficient of the regression model when the normalized trait is used (e.g., an effect size of 1.0 implies that each additional copy of the allele increases trait values by 1.0 standard deviation).

focused analysis on the basis of their cAMP specificity and reported expression in thyroid tissues. We have calculated the corresponding FDRs[27] by taking into account all the 503 SNPs tested in the candidate-gene analysis. Notably, by this criterion, the q value for SNPs falling in the *THRB* and *GNAQ* genes reached the significant threshold of 0.05 (q = 0.036 and q = 0.050, respectively), suggesting a possible involvement in the variability of TSH levels.

Discussion

The *PDE8B* gene, encoding a high-affinity cAMP phosphodiesterase,[37] is inferred to modulate circulating TSH levels in three independent populations, with a combined p value of 1.9×10^{-20}. Of the five characterized *PDE8B* isoforms, the major isoform *PDE8B1* and minor isoforms *PDE8B2* and *PDE8B3* are abundantly expressed in the thyroid.[16,38] Because *PDE8B* is undetectable in the pituitary,[39] we infer it to act primarily in the thyroid to catalyze the hydrolysis and inactivation of cAMP after TSH signaling. PDE8B could then influence TSH levels by feedback from an effect on the generation of T4 and T3 in the thyroid, in line with reports that both thyroglobulin endocytosis and thyroid-hormone secretion are stimulated by TSH via a cAMP-dependent pathway.[3]

Although no signals other than *PDE8B* reached genome-wide significance (Table 5), several candidate genes showed suggestive evidence of association. These included two other cAMP-specific phosphodiesterases, *PDE4D*,[34,35] and, at a much lower level of significance, *PDE7B*.[36] It is relevant that the cAMP-specific PDE4 family has also been suggested to be involved in modulating the cAMP signal after TSH stimulation of thyrocytes.[34] Furthermore, some evidence for association was also detected in our GWAS in *PDE10A*, a phosphodiesterase with dual specificity for cyclic nucleotides and stimulated by cAMP.[19] Analyses using differential inhibition or transfection of the range of phosphodiesterases and their variants in thyrocytes should help to determine their contribution to cAMP levels.

As for other genes previously implicated in TSH homeostasis, the *thyroid-hormone receptor, β* (*THRB*)[20] and several G protein genes—in particular, *GNAQ*,[21] previously reported to be required for TSH-induced thyroid-hormone synthesis and release—implicated in the signal transduction of hormone receptors, also showed suggestive

association signals (Table 5). Finally, some evidence of association was also noticed in two thyroid-specific genes, *thyroglobulin* (*TG*) and *thyroid-stimulating hormone receptor* (*TSHR*),[33] and in *POU class 1 homeobox 1* (*POU1F1* [MIM 173110]),[40] a gene expressed in the pituitary and important for the regulation of a number of pituitary hormones.

We infer that much of the TSH-level variance that subtly affects normal and possibly pathological states seems to be exerted at the level of cAMP degradation. It is possible that genomic *PDE8B* mutations could be responsible for certain thyroid diseases—for example, for increased serum TSH occasionally observed in individuals with no evidence of thyroid autoimmunity or loss-of-function mutations in the thyroid-hormone- or TSH-receptor genes.[41] Because PDE8B has the highest affinity for cAMP of any known phosphodiesterase,[37] the enzyme would be active even at low concentrations of cAMP, and a change in its level or activity could have an especially marked effect on TSH signaling. Consistent with this notion, a mutation in *PDE8B* that leads to elevated cAMP levels has now been identified in a case of adrenal hyperplasia in Cushing syndrome (AIMAH [MIM 219080]).[42] Increased cAMP-degrading PDE8B activity has also been detected in autonomous thyroid adenomas, where it may represent a compensatory mechanism opposing the constitutive activation of the cAMP pathway.[34] Other reports have noted *PDE8B* upregulation in Alzheimer's disease (AD [MIM 104300]) brain[43] and pituitary adenomas,[39] as well as involvement in a model of modified insulin secretion.[44]

PDE family members have increasingly been implicated in the pathogenesis of a number of other diseases, including cardiovascular disorders, renal failure,[45] adrenocortical hyperplasia,[42,46] and a wide variety of inflammatory pathologies.[47] Selective isoform-specific PDE inhibitors are already employed to treat several diseases, and thus PDE8B may provide a pharmaceutical target for certain thyroid pathologies.

Supplemental Data

Five tables are available at http://www.ajhg.org/.

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Web Resources

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