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Meta-analysis of two genome-wide association studies identifies four genetic loci associated with thyroid function

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Thyroid hormones play key roles in cellular growth, development and metabolism. Although there is a strong genetic influence on thyroid hormone levels, the genes involved are widely unknown. The levels of circulating thyroid hormones are tightly regulated by thyrotropin (TSH), which also represents the most important diagnostic marker for thyroid function. Therefore, in order to identify genetic loci associated with TSH levels, we performed a discovery meta-analysis of two genome-wide association studies including two cohorts from Germany, KORA (*n* = 1287) and SHIP (*n* = 2449), resulting in a total sample size of 3736. Four genetic loci at *5q13.3*, *1p36*, *16q23* and *4q31* were associated with serum TSH levels. The lead single-nucleotide polymorphisms of these four loci were located within *PDE8B* encoding phosphodiesterase 8B, upstream of *CAPZB* that encodes the β-subunit of the barbed-end F-actin-binding protein, in a former 'gene desert' that was recently demonstrated to encode a functional gene (*LOC440389*) associated with thyroid volume, and upstream of *NR3C2* encoding the mineralocorticoid receptor. The latter association for the first time suggests the modulation of thyroid function by mineral corticoids. All four loci were replicated in three additional cohorts: the HUNT study from Norway (*n* = 1487) and the two German studies CARLA (CARLA, *n* = 1357) and SHIP-TREND (*n* = 883). Together, these four quantitative trait loci accounted for ∼3.3% of the variance in TSH serum levels. These results contribute to our understanding of genetic factors and physiological mechanisms mediating thyroid function.

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INTRODUCTION

Thyroid hormones [thyroxine (T4) and triiodothyronine (T3)] are essentially involved in metabolic regulation, and minor variations in serum thyroid hormone levels can influence the basal metabolic rate, protein synthesis, fat and carbohydrate metabolism and cellular response to catecholamines (1,2). Accordingly, serum thyroid hormone levels are tightly regulated by thyrotropin (TSH) from the pituitary gland (3). Serum TSH concentrations in healthy individuals show considerable inter-individual variation (4), and twin and family studies have indicated that 58–71% of this variation is genetically determined (5–7). Thus, it is plausible that inter-individual differences in regulatory mechanisms of the pituitary–thyroid axis are responsible for a large proportion of the inter-individual differences in TSH concentrations. However, the causal genes have not been well established so far (5–7). Until now, only two genetic loci associated with circulating TSH levels have been identified and convincingly replicated. The lead single-nucleotide polymorphisms (SNPs) of these two loci were located in *PDE8B* encoding phosphodiesterase (PDE) 8B (OMIM +603390) (8) and in *CAPZB* (9), which encodes the β-subunit of the barbed-end F-actin binding protein. These two loci account for only 2.3 and 1.0–1.3% of TSH variance, respectively, and it is very likely that other genes are involved in the regulation of TSH levels. To investigate this further, we performed a meta-analysis of two genome-wide association studies (GWASs) to identify additional genetic loci influencing serum TSH levels in two population-based samples (KORA and SHIP) and replicated the results in three further independent cohort studies (HUNT, CARLA and SHIP-TREND).

RESULTS

The discovery GWAS meta-analysis identified several genomic regions associated with TSH levels (Fig. 1). Of the 2 524 918 SNPs tested by regression analysis, three distinct genetic loci reached genome-wide significance: *5q13.3* with the lead SNP rs2046045 localized within *PDE8B* encoding PDE 8B (Fig. 2A), *1p36* with its lead SNP rs10917477 located the upstream of *CAPZB*, which encodes the β-subunit of the barbed-end F-actin binding protein (Fig. 2B), and *4q31* where the lead SNP rs10028213 was identified the upstream of *NR3C2*, which encodes the mineralocorticoid receptor (Fig. 2C). Furthermore, an additional locus exhibited association *P*-values of $<10^{-7}$ and thus showed the suggestive evidence of association: *16q23* with the lead SNP rs3813582 (Fig. 2D). The latter locus represents a former 'gene desert' 110-kb upstream of *MAF*. However, it was recently demonstrated that it is actually directly located the downstream of a functional gene (*LOC440389*) associated with thyroid volume (10). All 44 SNPs representing the four distinct genetic loci exhibiting *P*-values of $<10^{-7}$ are listed in Supplementary Material, Table S1. The associated quantile–quantile plot in Supplementary Material, Figure S1, shows no deviation from the expected *P*-value distribution. To validate the independence of the four loci associated with TSH levels in the discovery stage, the lead SNPs were analyzed together in a multiple linear regression model, where the associations remained significant and mostly unchanged, indicating the statistical independence of the four SNPs from each other. The distribution of the mean log TSH levels depending on the number of TSH increasing alleles for both discovery cohorts is shown in Supplementary Material, Table S2. Additional adjustment for the participant's thyroid peroxidase (TPO) antibody status in the regression model did not influence the *P*-values (Table 1), suggesting that the associations of the four SNPs with TSH levels are independent of the TPO antibody status.

Subsequently, the four locus-specific lead SNPs were taken forward for replication in HUNT, CARLA and SHIP-TREND. The significant associations of all four lead SNPs with TSH levels were confirmed by the three replication studies (Table 1, Supplementary Material, Table S3).

Finally, a combined meta-analysis including all the samples from the two discovery and the three replication cohorts was carried out, encompassing a total number of 7463 individuals. The *P*-values obtained for the lead SNPs rs2046045, rs10917477, rs10028213 and rs3813582 of the four addressed loci *5q13.3* (*PDE8B*), *1p36* (*CAPZB*), *4q31* (*NR3C2*) and *16q23* (*LOC440389*) were 2.79×10^{-27}, 1.54×10^{-8}, 2.88×10^{-10} and 5.63×10^{-10}, respectively. Since *PDE8B*, *CAPZB* and *NR3C2* showed the moderate-to-high heterogeneity measured by the I^2 quantity (11) in the combined meta-analysis, we additionally meta-analyzed the four lead SNPs using a random-effects model to take into account the between-study heterogeneity. Although rs10917477 and rs10028213 did not reach genome-wide significance using this model, all *P*-values remained highly significant (Table 1), whereas the two known TSH-associated loci *PDE8B* and *CAPZB* showed the highest quantity of heterogeneity. These four quantitative trait loci together accounted for ~3.3% of the variance in the TSH serum levels.

DISCUSSION

The present meta-analysis confirmed that the common genetic variation in *PDE8B* was associated with TSH levels in the general population as reported by a previous GWAS (8) and a study including pregnant women (12). In our discovery meta-analysis, rs4704397 located within the first intron of *PDE8B* exhibited a *P*-value of 1.28×10^{-8}. This result is in line with the study of Arnaud-Lopez *et al.* (8), which found the strongest significant associations between rs4704397 and TSH levels. In a meta-analysis, Taylor *et al*. (13) also described a pronounced association between the rs4704397 SNP in *PDE8B* and serum TSH levels. For the replication analyses, rs2046045, also located within the first intron of *PDE8B* and in strong linkage disequilibrium (LD) with rs4704397 ($r^2 = 0.94$), was selected as a proxy SNP for the latter due to its better genotyping efficiency. It also exhibited genome-wide significant *P*-values, in both the discovery analysis and the combined meta-analysis. *PDE8B* is encoding the cAMP-hydrolyzing PDE 8B, which is strongly expressed in the thyroid and exhibits the highest affinity for cAMP compared with all other known PDEs (10,12,14,15), thus representing a plausible candidate gene modulating the circulating TSH levels.

Furthermore, we replicated the association of *1p36* with circulating TSH levels. This locus was originally identified in the GWAS of Panicker *et al*. (9) to be genome wide significantly

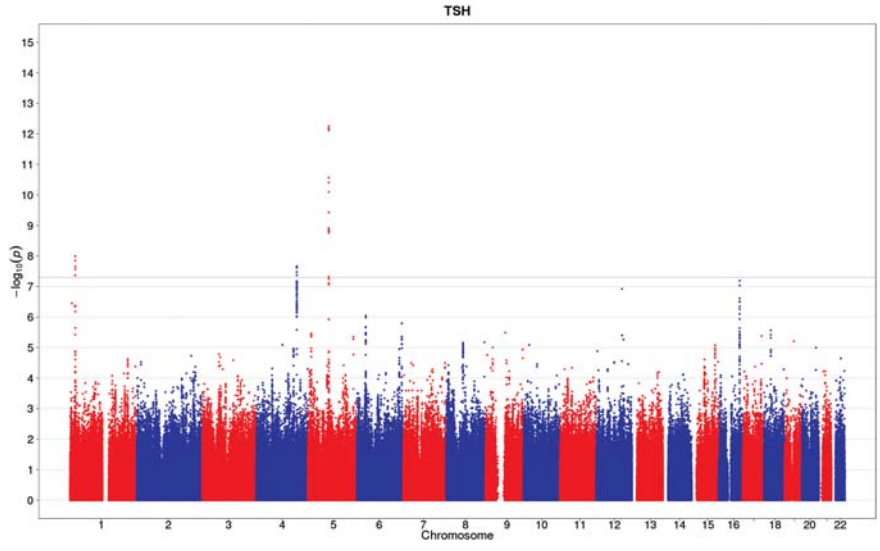

Figure 1. Manhattan plot showing that the significance of association of all SNPs in the meta-analysis with the log TSH levels. SNPs are plotted on the *x*-axis according to their position on each chromosome against association with the respective phenotype on the *y*-axis (shown as $-\log_{10}$ *P*-value). SNPs were filtered by the minor allele frequency of 1% and on an imputation quality value >0.3 in each cohort. The upper horizontal line indicates the threshold for genome-wide significance.

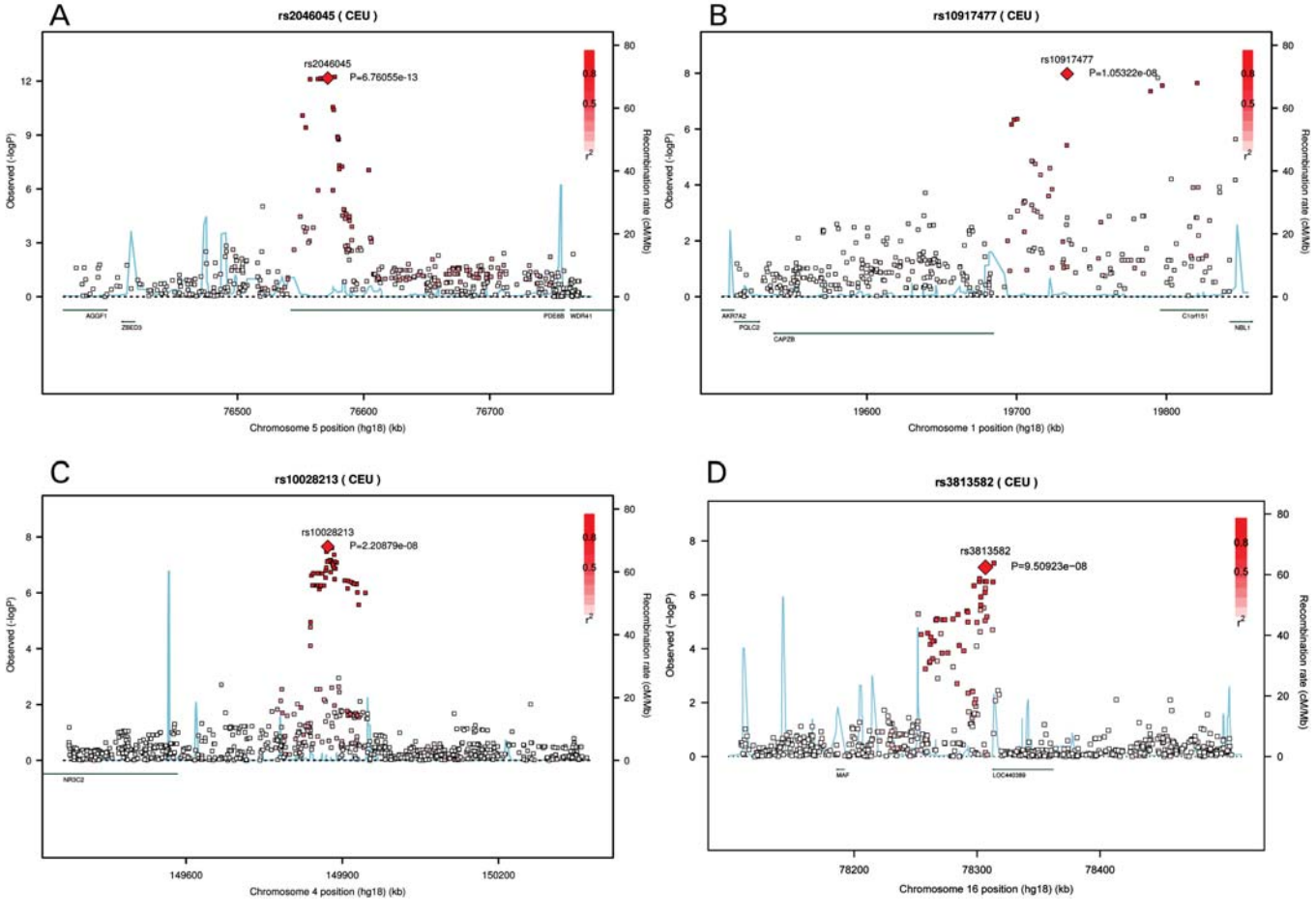

Figure 2. (**A**–**D**) Regional association plots showing the association signals in the regions of the four loci associated with the TSH levels on the $-\log_{10}$ scale as a function of the chromosome position in the meta-analysis: (A) the *PDE8B* locus on chromosome 5, (B) the locus upstream of *CAPZB* on chromosome 1, (C) the locus upstream region of *NR3C2* on chromosome 4 and (D) the *LOC440389* locus on chromosome 16. Large diamonds in red indicate the lead SNPs exhibiting the lowest *P*-values for association with the log TSH levels. The correlations (r^2) between each of the surrounding SNPs and the respective lead SNP are indicated by red shading. The left *y*-axis indicates the *P*-values for the association with log TSH levels, and the right *y*-axis indicates the estimated recombination rates (HapMap Phase III) given in light blue. Genes as well as the direction of transcription (NCBI) are displayed by green bars.

Table 1. Association between TSH and the four replicated SNPs in each stage of the meta-analysis

SNP	Chr	Position	Gene	Effect allele	Other allele	Effect allele frequency	Variance explained
rs2046045	5	76571567	***PDE8B***	T	G	61.8%	1.82%
Stage	Estimate	SE	*P*-value	*P*-value (adjusted TPOab)	*P*-value (random effect)	Heterogeneity I^2	*N*
Discovery	−0.111	0.015	6.76E−13	6.51E−13	–	78	3735
Replication	−0.119	0.015	3.08E−16	–	–	44	3717
Combined	−0.115	0.011	2.79E−27	–	1.78E−14	51	7452
SNP	Chr	Position	Gene	Effect allele	Other allele	Effect allele frequency	Variance explained
rs10917477	1	19733693	*(CAPZB)*	A	G	51.1%	0.42%
Stage	Estimate	SE	*P*-value	*P*-value (adjusted TPOab)	*P*-value (random effect)	Heterogeneity I^2	*N*
Discovery	−0.084	0.015	1.05E−08	1.10E−08	–	0	3691
Replication	−0.033	0.014	1.11E−02	–	–	0	3700
Combined	−0.058	0.010	1.54E−08	–	1.89E−04	51	7391
SNP	Chr	Position	Gene	Effect allele	Other allele	Effect allele frequency	Variance explained
rs10028213	4	149872060	*(NR3C2)*	C	G	82.3%	0.59%
Stage	Estimate	SE	*P*-value	*P*-value (adjusted TPOab)	*P*-value (random effect)	Heterogeneity I^2	*N*
Discovery	0.106	0.019	2.21E−08	9.12E−09	–	51	3715
Replication	0.062	0.019	4.40E−04	–	–	0	3697
Combined	0.084	0.013	2.88E−10	–	8.43E−07	38	7412
SNP	Chr	Position	Gene	Effect allele	Other allele	Effect allele frequency	Variance explained
rs3813582	16	78306854	*(LOC440389) (MAF)*	T	C	68.1%	0.48%
Stage	Estimate	SE	*P*-value	*P*-value (adjusted TPOab)	*P*-value (random effect)	Heterogeneity I^2	*N*
Discovery	0.085	0.016	9.51E−08	2.17E−08	–	0	3697
Replication	0.052	0.015	2.64E−04	–	–	0	3717
Combined	0.068	0.011	5.63E−10	–	5.46E−10	0	7414

Effect size estimates and corresponding standard errors (SE) are given for each copy of the effect allele and are expressed as the natural logarithm of TSH. For each SNP, the direction of effect was consistent between all cohorts. If the SNP is located within a gene, the corresponding gene is given in bold font; in any other case, the nearest known gene is written in parenthesis. Positions refer to NCBI build 36. The *P*-value of the replication stage is one-sided, and all other *P*-values are two-sided.

TSH-level associated (*P*-value: 3.2×10^{-8}), with the lead SNP rs10917469 located the upstream of *CAPZB*. The same locus exhibited genome-wide significance in the present study (Fig. 2B), with the minor allele G (48.9%) of the lead SNP rs10917477 of the combined analysis being in moderate LD with the minor allele G (15.3%) of the lead SNP rs10917469 described by Panicker *et al*. ($r^2 = 0.15$, $D' = 1$). However, in contrast to the decreasing effect on TSH levels per minor allele of rs10917469 in the study of Panicker *et al*., the minor allele of our lead SNP rs10917477 was associated with increased TSH levels. As in both studies, the associated locus is located the upstream of *CAPZB*; it is tempting to hypothesize that the causative polymorphism(s) underlying the observed associations might influence the activity of the *CAPZB* promoter, thus modifying the expression of the gene.

A GWAS that was recently published by our group demonstrated the genome-wide significant association of the same locus upstream of *CAPZB* with thyroid volume and goiter (10), as illustrated by the fact that the lead SNPs for these two phenotypes, rs12138950 (minor allele C, 15.3%) and rs10917468 (minor allele C, 21.7%), are in moderate LD with rs10917477, the lead SNP for TSH levels in the present analysis ($r^2 = 0.15$ and 0.26, respectively; $D' = 1$ in both cases), and in perfect or pronounced LD with rs10917469, the lead SNP in the Panicker *et al.* study ($r^2 = 1.00$ and 0.60, respectively; $D' = 1$ in both cases). Despite the opposite effects of the minor alleles of rs10917477 and rs10917469 on TSH levels that might indicate a complex local haplotype substructure, both SNPs are consistently associated with antipodal effects on thyroid volume and TSH levels: the minor alleles of rs10917477 and rs10917469 are associated with the decreased and increased thyroid volumes, respectively ($P = 7.5 \times 10^{-14}$ and 1.1×10^{-16}), according to the results of Teumer *et al.* (10). The molecular mechanism that may underlie these observed associations has been discussed in detail (10). In particular, it was hypothesized that the rs12138950 and rs10917468 minor allele-associated haplotype specifies an enhanced *CAPZB* promoter activity, resulting in an increased amount of the non-muscle-specific β2-subunit of the capping protein (CP) in the thyroid cells. According to the regulatory model proposed in Teumer *et al.* (10), the resulting increased thyroidal amounts of CP mediate the alleviated reception of the incoming TSH/cAMP signal as a result of attenuated actin barbed-end uncapping activity. This should cause reduced thyroglobulin engulfment by filopodia and decreased T3/T4 release from the thyroid cells and, in turn, compensatory thyroid hyperplasia. After the final cessation of its growth, the enlarged thyroid is predicted to produce amounts of T3 and T4 that are even above the physiological threshold values, and negative feedback regulation via these increased levels subsequently causes a reduced TSH production and secretion until the thyroid hormone levels are again within the physiological range. As indeed observed, the newly established equilibrium should be characterized by an increased thyroid volume and decreased TSH levels. Since TSH is an important stimulator of thyroid growth, the

increased thyroid volume associated with the decreased TSH level might therefore seem contra-intuitive. Nevertheless, this negative association between both logarithmic measurements was also observed in the SHIP population ($r = -0.37$, $P < 2 \times 10^{-16}$). A second, independent association of the *16q23* locus with thyroid volume and goiter that was reported to be located within the *CAPZB* genic region (10) showed only a weak association with TSH levels in the discovery stage in the present study. The minor allele G (33.7%) of the corresponding lead SNP rs12045440 that was associated with both decreasing thyroid volume and reduced risk for goiter exhibited a nominal significant association with elevated TSH levels at $P = 0.0013$.

In the present study, the locus at *16q23*, which was also identified in a GWAS to be associated with thyroid volume and goiter (10), showed the suggestive evidence of association with TSH levels in the discovery stage and reached genome-wide significance in the combined sample set. Although the association missed genome-wide significance in the discovery stage, the successful replication and the fact that the corresponding lead SNPs rs17767419 for thyroid volume and rs3813579 for goiter are in perfect or pronounced LD with the lead SNP of the TSH level, rs3813582 ($r^2 = 1.00$ and 0.40; $D' = 1.0$ and 0.94, respectively), evidence that this locus might be truly associated with TSH levels. Furthermore, the D' values close to 1 suggest one common haplotype underlying the associations with all three phenotypes, but fine mapping in a larger sample and functional analyses is necessary to reinforce this association and to give further insights into the underlying genetically driven causalities. Finally, again comparable with the locus upstream of *CAPZB*, the minor allele C (31.9%) of the lead SNP was associated with decreased TSH levels and an increased thyroid volume.

All three lead SNPs at *16q23* are located within a region of 4.8 kb immediately downstream of the formerly predicted gene *LOC440389* that meanwhile has been removed from the NCBI database as a result of the standard genome annotation processing (10). Therefore, this chromosomal region represented a 'gene desert' with the next annotated gene *MAF* in a distance of 121 kb to *LOC440389*. However, the presence of the correctly spliced mature *LOC440389* mRNA as predicted by the deleted database entry was recently demonstrated in thyroid tissue, and it was shown that this transcript is clearly more abundant in thyroid tissue when compared with skeletal muscle tissue (10). These results proved that *LOC440389* represents a real gene and that its withdrawal from the NCBI database was incorrect. As the minor allele of the associated lead SNPs are localized within a region of marked LD encompassing the 3′-end of *LOC440389* and the region immediately downstream, it might be hypothesized that the putative causative polymorphism(s) affecting thyroid size and TSH levels influences *LOC440389* expression by modifying its 3′-trailer sequence. Strikingly, microRNA (miRNA) target sites are frequent in 3′-untranslated regions (3′-UTRs) of human protein-coding genes, and it has been proposed that miRNA regulation may be affected by sequence variants in 3′-UTRs (10).

How the predicted 85-amino-acid protein encoded by *LOC440389* influences TSH levels and thyroid volume is unknown. Database searches demonstrated, apart from the *Pan troglodytes* ortholog encoded by *LOC454261* (100% sequence homology), no further protein showing at least partial marked sequence homology to the derived *LOC440389* gene product. However, the fact that the association pattern is comparable with that of the locus upstream of *CAPZB* (thyroid volume increasing the allele is associated with decreased TSH levels) might point toward a similar underlying mechanism: increased or decreased amounts of the *LOC454261* gene product—this is currently not clear—mediated by the minor allele-associated haplotype might cause the poorer reception of the incoming TSH/cAMP signal when compared with the major allele-associated haplotype due to a yet unknown mechanism and, as the consequence, compensatory thyroid hyperplasia. According to the model described for the *CAPZB* locus, the enlarged thyroid will subsequently produce T3 and T4 in amounts even above the physiological threshold values, and negative feedback regulation mediated by these increased thyroid hormone levels subsequently causes a reduced TSH production. In the final equilibrium, an increased thyroid volume would be accompanied by decreased TSH levels, as observed in the present study.

The described association of SNPs with both an increased thyroid volume and decreased TSH levels became moreover apparent in the case of the *15q21* locus accounting for the remaining association on thyroid volume previously reported by our group (10). The minor C allele (24.9%) of the corresponding lead SNP rs4338740 that was associated with increasing thyroid volume (1.4×10^{-12}) and goiter risk (2.8×10^{-13}) in that study was also associated with decreasing TSH levels in the present work (discovery stage: $\beta = -0.07$, $P = 4.2 \times 10^{-5}$). The SNP is located within *FGF7* encoding the fibroblast growth factor 7. As discussed in Teumer *et al.* (10), it can be predicted that the causative sequence variant underlying this association causes an enhanced FGF7 signal and thus mediating a more pronounced proliferation of thyroid cells in risk allele carriers, which finally results in the already described equilibrium of decreased TSH levels and an increased thyroid volume.

One of the most interesting findings of the present study is the detection of a genome-wide significant association between TSH levels and a locus at *4q31* upstream of *NR3C2*. The present GWAS is the first describing an association between this locus and thyroid function, where the minor allele G (17.7%) of the lead SNP rs10028213 is associated with increased TSH levels. The *NR3C2* encoded mineralocorticoid receptor generally mediates the actions of aldosterone, which represents the major mineralocorticoid. The protein functions as a transcription factor that, after being activated by hormone binding, translocates as a dimer from the cytoplasm to the nucleus. Subsequently, the receptor recognizes mineralocorticoid response elements in order to transactivate the expression of its target genes, thereby mediating the aldosterone effects on salt and water balance by inducing target gene expression. As the detected locus is clearly located in the upstream region of *NR3C2*, it is obvious to assume that the haplotype responsible for the observed association modifies the promoter activity of the gene; however, at present, it is not clear if stronger or weaker *NR3C2* expression causes increased TSH levels.

The question arises how the mineralocorticoid receptor might influence TSH levels. This could be due to non-direct effects: mineralocorticoid receptors in the hippocampus play important roles in the control of the hypothalamic–pituitary–adrenal (HPA) axis (13). Glucocorticoids, which represent the end products of HPA axis activation, maintain the basal HPA activity and have been suggested to be involved in hypothalamic–pituitary–thyroid axis regulation. Indeed, glucocorticoids inhibit pituitary gonadotropin, growth hormone and TSH secretion, render the target tissues of sex steroids and growth factors resistant to these signaling molecules and suppress 5′ deiodinases, which converts the relatively inactive T4 to T3, further contributing to the suppression of reproductive, growth and thyroid functions (16,17). Thyroid hormones, on their part, reduce the efficacious inactivation of cortisol by its conversion to cortisone; impaired cortisol inactivation results in hypertension and hypokalemia characterized by an excess of mineralocorticoids (18).

Alternatively, the observed effects of genetic polymorphisms that most probably modify the expression strength of *NR3C2* might be more directly mediated: strikingly, according to the BioGPS database, the amounts of *NR3C2*-specific mRNA in the thyroid gland are remarkably high; indeed, together with that in colon and prefrontal cortex, thyroidal *NR3C2* expression represents the strongest at all when compared with all other organs and cell types available in this database. Therefore, it might be hypothesized that the mineralocorticoid receptor positively regulates the expression of thyroidal genes involved in the reception and the transduction of the incoming TSH/cAMP signal. Then, depending on the genotype-specific numbers of mineralocorticoid receptor molecules in the thyroid cells, different amounts of thyroid hormones would be predicted to be produced and secreted. In turn, via negative feedback regulation, these would cause different genotype-specific TSH levels, as observed in this study. The identification of putative aldosteron-regulated genes in the thyroid should therefore represent one important focus of future follow-up analyses. Additionally, further studies are needed to replicate the present finding.

In conclusion, the present study identified four quantitative trait loci associated with TSH levels in the general population. The identification of primary genetic determinants of thyroid function may not only enhance our understanding of thyroid–pituitary axis set points, but also provides a focus for several novel research avenues.

MATERIALS AND METHODS

The two GWASs meta-analyzed in the discovery study included 1287 probands aged 32–79 from the Cooperative Health Research in the Region of Augsburg Study (KORA F4, Southern Germany) and 2449 participants aged 25–88 from the Study of Health in Pomerania (SHIP-1, North-East Germany), resulting in a total sample size of 3736. All individuals were of European ancestry. Approval was obtained by the local ethic committees for both studies, and informed consent was obtained from the study participants. For replication, 883 participants of the SHIP-Trend study, a new and independent population survey recruited from the SHIP study region in North-East Germany, 1357 probands from the CARLA (Cardiovascular Disease, Living and Ageing in Halle) Study, another population-based study from Central Germany, and 1487 individuals from a Norwegian population-based study (Nord-Trøndelag Health Study, HUNT) were analyzed. The baseline characteristics of the cohort studies as well as the thyroid hormone measurement methods are described in Table 2.

Genotyping procedures and quality controls of the cohorts contributing *in silico* genotype data (KORA F4, SHIP-1 and SHIP-TREND) are described in detail in Supplementary Material, Table S4. Individuals taking thyroid medication or medications likely to influence thyroid function (e.g. anticonvulsants or oral glucocorticoids), persons with biochemical evidence of clinically significant thyroid dysfunction or reporting known thyroid disorders as well as women pregnant at the time of examination were excluded from the analysis, leaving 3736 participants for the discovery meta-analysis.

All analyses were adjusted for age, gender, body surface area and current smoking (yes/no) and were carried out using the software SNPTEST (19) and QUICKTEST by taking the uncertainties of the imputed genotypes into account. Associations were considered genome-wide significantly below a P-value of 5×10^{-8} which corresponds to a Bonferroni correction for the estimated one million independent common variant tests in the human genome of European individuals (20). Only SNPs with available association data from both contributing studies were included in the discovery meta-analysis. All SNPs with a minor allele frequency <0.01 or having an imputation quality <0.3 (as measured by the observed/expected variance ratio) in any of the two cohorts were removed from subsequent analyses. In total, 216 979 SNPs were excluded by these criteria, and 2 524 918 directly genotyped or imputed autosomal SNPs remained for analysis. Associations were tested using a linear additive model on natural log-transformed TSH levels. Meta-analysis of the individual results were performed using a fixed-effect inverse-variance weighted model as implemented in the METAL package (21). Genomic control correction was applied to individual cohorts' GWAS results as well as to the meta-analysis GWAS results ($\lambda = 1.004$), if the corresponding genomic control factor was >1. An exact χ^2 test was used to detect deviations from the Hardy–Weinberg equilibrium. All SNPs found to be associated with thyroid function in the discovery meta-analysis were in the Hardy–Weinberg equilibrium ($P > 0.001$). Among the strongest TSH-level-associated loci having association P-values of $<10^{-7}$, those SNPs that exhibited the smallest P-value and a genotyping efficiency $>95\%$ if directly genotyped on the array (two SNPs substituted) were selected as lead SNPs and taken forward into replication.

For replication, selected SNPs were *de novo* genotyped in the HUNT and CARLA studies. Details of examination procedures, genotyping and quality criteria are described in the original studies (22–24) and in Supplementary Material. The SHIP-TREND sample representing the third replication cohort was individually genotyped using the Illumina Human Omni 2.5 genotyping array, and the generated data were imputed against the HapMap v22 CEU reference panel using the software IMPUTE 2.1.2.3. Details are given in Supplementary Material. SNPs were considered as replicated if their one-sided association P-value was <0.0125, which

Table 2. Description of the GWA study population and the replication samples

Cohort characteristics	Study name Cooperative health research in the region of Augsburg, survey F4	Study of health in Pomerania, SHIP-1	Cardiovascular disease, living and ageing study	The Nord-Trøndelag health study	Study of health in Pomerania, TREND
Study acronym	KORA F4	SHIP-1	CARLA	HUNT	SHIP trend
Study design	Population-based	Population-based	Population-based	Population-based	Population-based
Sample size	1287	2449	1357	1487	883
Age in years (range)	60 (32–79)	53 (25–88)	64.1 (45 -83)	49 (20–79)	49.5 (20–81)
Females (%)	541 (41.9)	1221 (49.8)	523 (38.5)	747 (50.2)	474 (53.68)
Current smokers (%)	210 (16.29)	645 (26.34)	281 (20.71)	452 (30.4)	194 (21.97)
Bovine serum albumin in m^2 (SD)	1.90 (0.21)	1.90 (0.21)	1.90 (0.20)	1.89 (0.20)	1.89 (0.20)
Serum TSH in mU/l (SD)	1.50 (1.47)	0.96 (0.91)	0.9 (0.82)	1.85 (1.98)	1.34 (0.82)
TSH measurement	Measurement by immunochemiluminescent procedures (Dimension Vista System, Siemens)	Immulite 2000, third generation (Diagnostic Products Corporation, DPC, Los Angeles, USA)	Flex reagent cartridge (Dade Behring, Marburg, Germany); non-fasting	DELFIA hTSH Ultra (Wallac Oy, Turku, Finland)	Measurement by immunochemiluminescent procedures (Dimension Vista System, Siemens)
Measurement of autoantibodies to thyroperoxidase (TPO)	Enzyme immunoassay (VARELISA, Elias Medizintechnik, GmbH, Freiburg, Germany); TPOAB levels of >200 IU/ml were regarded as positive; functional sensitivity of this assay was 1 IU/ml	Enzyme immunoassay (VARELISA, Elias Medizintechnik, GmbH); TPOAB levels of >200 IU/ml were regarded as positive; functional sensitivity of this assay was 1 IU/ml			
Sample exclusions by phenotype	Individuals taking thyroid medication or reporting thyroid disorders, women pregnant at the time of sample collection (except SHIP trend)				

corresponds to a Bonferroni-corrected 5% significance level of the four independent loci. Using the described sample size, the power for replication of these SNPs was >99%. Power calculation was done using the software QUANTO 1.2.4. TPO antibody-adjusted meta-analysis was computed using R, and the random-effects model was calculated using the package metaphor (25).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG online.

Conflict of Interest statement. None declared.

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