Human Molecular Genetics, 2014, Vol. 23, No. 16 **4433–4442**
doi:10.1093/hmg/ddu145
Advance Access published on April 9, 2014

A genome-wide association study on thyroid function and anti-thyroid peroxidase antibodies in Koreans

Soo Heon Kwak[1,†,‡], Young Joo Park[1,2,†,‡], Min Jin Go[5,†,‡], Kyu Eun Lee[3], Su-jin Kim[3], Hoon Sung Choi[1], Tae Hyuk Kim[1], Sung Hee Choi[2,6], Soo Lim[2,6], Ki Woong Kim[4,7,8], Do Joon Park[1,2], Sung Soo Kim[5], Jong-Young Lee[5,¶], Kyong Soo Park[1,2,9,¶], Hak C. Jang[2,6,¶] and Nam H. Cho[10,¶,*]

[1]Department of Internal Medicine, Seoul National University Hospital, Seoul, Korea, [2]Department of Internal Medicine, [3]Department of Surgery, and [4]Department of Psychiatry, Seoul National University College of Medicine, Seoul, Korea, [5]Center for Genome Science, Korea National Institute of Health, Osong Health Technology Administration Complex, Chungcheongbuk-do, Korea, [6]Department of Internal Medicine, and [7]Department of Psychiatry, Seoul National University Bundang Hospital, Seongnam, Korea, [8]Department of Brain and Cognitive Science, Seoul National University College of Natural Sciences, Seoul, Korea, [9]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Korea and [10]Department of Preventive Medicine, Ajou University School of Medicine, Suwon, Korea

Received December 30, 2013; Revised March 18, 2014; Accepted March 28, 2014

Genetic factors are thought to be an important determinant of thyroid function and autoimmunity. However, there are limited data on genetic variants in Asians. In this study, we performed a genome-wide association study on plasma thyroid-stimulating hormone (TSH) and free thyroxine (fT_4) concentration and anti-thyroid peroxidase (anti-TPO) antibody positivity in 4238 Korean subjects. In the Stage 1 genome scan, 3396 participants from the Ansung cohort were investigated using 1.42 million genotyped or imputed markers. In the Stage 2 follow-up, 10 markers were genotyped in 842 participants from the Korean Longitudinal Study on Health and Aging cohort. An intronic variant in *VAV3,* rs12126655, which has been reported in Europeans, was significantly associated with plasma TSH concentration in the joint Stages 1 and 2 analyses ($P = 2.2 \times 10^{-8}$). We observed that a novel variant, rs2071403, located 75 bp proximal to the translational start site of *TPO* was significantly associated with plasma anti-TPO antibody positivity in the joint Stages 1 and 2 analyses ($P = 1.3 \times 10^{-10}$). This variant had a marginal sex-specific effect, and its association was more significant in females. Subjects possessing the rs2071403A allele, associated with an absence of the anti-TPO antibody, had decreased TPO mRNA expression in their thyroid tissue. Another intronic variant of *HLA-DPB2*, rs733208, had a suggestive association with anti-TPO antibody positivity ($P = 4.2 \times 10^{-7}$). In conclusion, we have identified genetic variants that are strongly associated with TSH level and anti-TPO antibody positivity in Koreans. Further replications and meta-analysis are required to confirm these findings.

INTRODUCTION

Thyroid hormone has diverse physiologic functions, including fetal development, oxygen consumption, thermogenesis and glucose and lipid metabolism (1,2). Within an individual, thyroid hormone and thyroid-stimulating hormone (TSH) levels are maintained within a relatively constant range over a

*To whom correspondence should be addressed at: Department of Preventive Medicine, Ajou University School of Medicine, 164, World Cup-ro, Yeongtong-gu, Suwon 443–721, Korea. Tel: +82 312195900; Fax: +82 312195901; Email: chnaha@ajou.ac.kr
†These authors contributed equally to this work.
‡The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint First Authors.
¶The authors wish it to be known that, in their opinion, the last four authors should be regarded as joint Senior Authors.

long period (low intra-individual variation) (3). However, the variation between different individuals is larger (high inter-individual variation) (3). Inter-individual variability is partially explained by genetic factors. In a recent study, the heritability of free thyroxine (fT_4) and TSH was 89 and 49%, respectively (3). The advent of genome-wide association studies (GWAS) have enabled us to identify the genetic variants associated with TSH and fT_4 concentrations (4–10). A large-scale meta-analysis of GWAS in Europeans confirmed 20 genetic variants associated with TSH and six variants associated with fT_4 concentrations. However, the genetic association of TSH and fT_4 in Asians is not fully understood.

The dysregulation of thyroid hormone, resulting in hypo- or hyperthyroidism, is a relatively common condition. The most common clinical problem is subclinical hypothyroidism (defined as an increased TSH level with normal fT_4 concentration), which occurs in 4.3% of the US population (11) and 11.7% of the Korean population (12). A high concentration of anti-thyroid peroxidase (TPO) antibody is a well-known risk factor associated with the development of thyroid dysfunction (13). Anti-TPO antibody is observed in nearly all patients with Hashimoto's thyroiditis (also known as chronic lymphocytic thyroiditis). The presence of this antibody suggests the pathogenic role of autoimmunity in the development of Hashimoto's thyroiditis. Several studies have investigated genetic variants associated with Hashimoto's thyroiditis in Europeans (10,12). The genetic association of anti-TPO antibody positivity has not been investigated on a genome-wide scale.

The aim of this study was to investigate the genetic variants associated with plasma TSH and fT_4 concentrations and anti-TPO antibody positivity in Koreans. We performed a two-staged GWAS in 4238 Korean participants recruited from two independent community-based cohorts.

RESULTS

Stage 1 genome scan

The participants in the Stage 1 genome scan were from the Ansung cohort comprising the Korean Genome Epidemiology Study (KoGES). The clinical characteristics of the participants are shown in Table 1. To test for an association between the genetic variants and TSH and fT_4 concentrations, we performed a linear regression analysis adjusting for age and sex in 2789 participants. For anti-TPO antibody positivity, we used a logistic regression adjusting for age and sex in a case-controlled manner of 3396 participants. A total of 351 669 single-nucleotide polymorphism (SNP) variants were actually genotyped and passed our stringent quality control filters. After the imputation, we were able to use 1 418709 SNPs for analyses. The quantile–quantile plots and Manhattan plots from the association tests are shown in the Supplementary Material, Figure S1. Ten independent variants were selected for suggestive associations according to our predefined threshold of $P < 1.0 \times 10^{-5}$, except for rs17111090 ($P = 2.4 \times 10^{-3}$) in *TRHDE* (thyrotropin-releasing hormone degrading enzyme) which was selected based on its biological plausibility (Table 2). The full list of variants that showed associations with $P < 1.0 \times 10^{-5}$ are listed in the Supplementary Material, Tables S1–S3. To eliminate hidden population stratification and cryptic relatedness, a variance component approach using EMMAX (http://www.sph.umich.edu/csg/kang/emmax/) was used to test the associations (Supplementary Material, Tables S1–S3) (14). The EMMAX association results were similar to the original analyses.

Stage 2 follow-up and joint Stages 1 and 2 analyses

The 10 variants were further evaluated in the Stage 2 analysis. The 829 participants for the Stage 2 follow-up were from the Korean Longitudinal Study on Health and Aging (KLoSHA) cohort, and their clinical characteristics are shown in Table 1. Among the 10 variants, three showed significant associations ($P < 0.05$) (Table 2). The rs12126655 variant in the intron of *VAV3* (*vav 3 guanine nucleotide exchange factor*) was associated with TSH concentration ($P = 3.7 \times 10^{-3}$). The rs2071403 variant in the 5′ untranslated region (UTR) of *TPO* (*thyroid peroxidase*) and rs733208 in the intron of *HLA-DPB2* (*major histocompatibility complex, class II, DP beta 2* (*pseudogene*)) were associated with anti-TPO antibody positivity ($P = 2.7 \times 10^{-4}$ and 3.0×10^{-2}). In the joint Stages 1 and 2 meta-analyses, we observed a genome-wide significance for an association between the rs12126655 variant in *VAV3* and TSH concentration ($P = 2.2 \times 10^{-8}$) and the rs2071403 variant in *TPO* and anti-TPO antibody positivity ($P = 1.3 \times 10^{-10}$). The rs12126655 variant was in strong linkage disequilibrium (LD) [$r^2 = 0.84$ in HapMap Han Chinese in Beijing, China (CHB) and Japanese in Tokyo, Japan (JPT)] with rs17020124, which was recently reported to be associated with TSH concentration in Europeans (8). Regional association plots of the variants near rs12126655 and rs2071403 are shown in Figure 1. Two additional variants showed a suggestive genome-wide significant association with fT_4 (rs7951195 in 11p12, $P = 4.9 \times 10^{-7}$)

Table 1. Clinical characteristics of study participants

	Stage 1 genome scan (Ansung cohort)			Stage 2 follow-up (KLoSHA cohort)		
	Men	Women	*P*	Men	Women	*P*
N (%)	1527 (45.0%)	1869 (55.0%)		362 (43.0%)	480 (57.0%)	
Age (years)[a]	56.1 ± 8.7	55.8 ± 8.9	0.100	76.5 ± 8.9	76.7 ± 9.1	0.715
TSH (μIU/L)[b]	1.62 (1.08–2.34)	2.36 (1.53–3.51)	<0.001	2.56 (1.77–3.67)	2.66 (1.70–3.74)	0.815
fT_4 (ng/dl)[b]	0.99 (0.91–1.07)	0.97 (0.90–1.05)	0.001	1.24 (1.06–1.45)	1.15 (0.94–1.36)	<0.001
Positive anti-TPO antibody, *N* (%)[b]	108 (7.1%)	269 (14.4%)	<0.001	32 (9.0%)	76 (15.9%)	0.003

[a]Data are shown as mean ± SD and *t*-test was used to compare means between men and women.
[b]Data are shown as median (interquartile range) and Mann–Whitney *U* test was used to compare means between men and women.

Table 2. Association between genetic variants and TSH, fT_4 concentration and anti-TPO antibody positivity

Variant	Chr	Position (bp)	A1	Nearby gene	Stage 1 genome scan					Stage 2 follow-up				
					n	MAF	β	SE	*P*	*n*	MAF	β	SE	*P*
TSH concentration														
rs12126655	1	108 158 343	G	*VAV3*	2665	0.35	0.135	0.028	1.7×10^{-6}	834	0.36	0.147	0.051	3.7×10^{-3}
rs17042140	1	212 741 548	T	*PTPN14*	2750	0.46	0.129	0.026	6.6×10^{-7}	833	0.43	0.019	0.049	7.0×10^{-1}
rs11026407	11	22 061 606	C	*ANO5*	2640	0.26	−0.149	0.030	6.1×10^{-7}	832	0.28	−0.038	0.057	5.0×10^{-1}
rs1203867	20	22 479 141	T	*FOXA2*	2607	0.13	−0.175	0.039	9.0×10^{-6}	830	0.17	−0.023	0.065	7.2×10^{-1}
FT_4 concentration														
rs6834538	4	113 763 601	T	*C4orf21*	2754	0.03	−0.351	0.074	2.4×10^{-6}	826	0.04	−0.117	0.133	3.8×10^{-1}
rs7785730	7	28 062 724	G	*JAZF1*	2787	0.48	−0.122	0.027	4.6×10^{-6}	823	0.49	−0.050	0.049	3.1×10^{-1}
rs7951105	11	38 988 369	T	–	2771	0.15	−0.181	0.036	6.8×10^{-7}	827	0.17	−0.092	0.068	1.8×10^{-1}

						MAF						MAF				
					n	Cases	Controls	OR	SE	*P*	N	Cases	Controls	OR	SE	*P*
Anti-TPO antibody positivity																
rs2071403	2	1 396 251	A	*TPO*	3391	0.27	0.38	0.626	0.087	8.6×10^{-8}	827	0.27	0.40	0.541	0.169	2.7×10^{-4}
rs733208	6	33 190 286	A	*HLA-DPB2*	3300	0.39	0.30	1.450	0.081	4.6×10^{-6}	825	0.38	0.31	1.385	0.151	3.0×10^{-2}
rs17111090	12	71 035 409	A	*TRHDE*	3378	0.03	0.01	2.144	0.251	2.4×10^{-3}	829	0.00	0.01	0.754	1.062	7.9×10^{-1}

Variant	Chr	Position (bp)	A1	Nearby gene	Joint stage 1 and 2					
					n	β	SE	*P*	I^2	Het *P*
TSH concentration										
rs12126655	1	108 158 343	G	*VAV3*	3499	0.138	0.025	2.2×10^{-8}	0	0.837
rs17042140	1	212 741 548	T	*PTPN14*	3583	0.105	0.023	4.4×10^{-6}	75	0.048
rs11026407	11	22 061 606	C	*ANO5*	3472	−0.125	0.026	2.1×10^{-8}	66	0.085
rs1203867	20	22 479 141	T	*FOXA2*	3437	−0.134	0.034	6.7×10^{-5}	75	0.045
FT_4 concentration										
rs6834538	4	113 763 601	T	*C4orf21*	3580	−0.296	0.065	5.2×10^{-6}	58	0.124
rs7785730	7	28 062 724	G	*JAZF1*	3610	0.106	0.023	6.1×10^{-6}	41	0.195
rs7951105	11	38 988 369	T	–	3598	−0.161	0.032	4.9×10^{-7}	25	0.250

					n	OR	SE	*P*	I^2	Het *P*
Anti-TPO antibody positivity										
rs2071403	2	1 396 251	A	*TPO*	4218	0.607	0.078	1.3×10^{-10}	42	0.190
rs733208	6	33 190 286	A	*HLA-DPB2*	4125	1.435	0.071	4.2×10^{-7}	0	0.530
rs17111090	12	71 035 409	A	*TRHDE*	4207	2.029	0.244	3.8×10^{-3}	65	0.092

Associations were tested with minor alleles by linear regression for TSH and fT_4 concentration and by logistic regression for anti-TPO antibody positivity. For TSH and fT_4 concentration, the effect size is shown as β and for anti-TPO antibody positivity as odds ratio (OR). The minor allele and its physical position are indexed to the positive strand of the National Center for Biotechnology Information (NCBI) build 36. The nearby gene is defined as the gene nearest to the variant within the boundary of the 50-kb distance. Chr, chromosome number; A1, minor allele; *N*, number of subjects; MAF, minor allele frequency; β, effect size of linear regression; SE, standard error; I^2, heterogeneity estimate; Het *P*, *P*-value for heterogeneity.

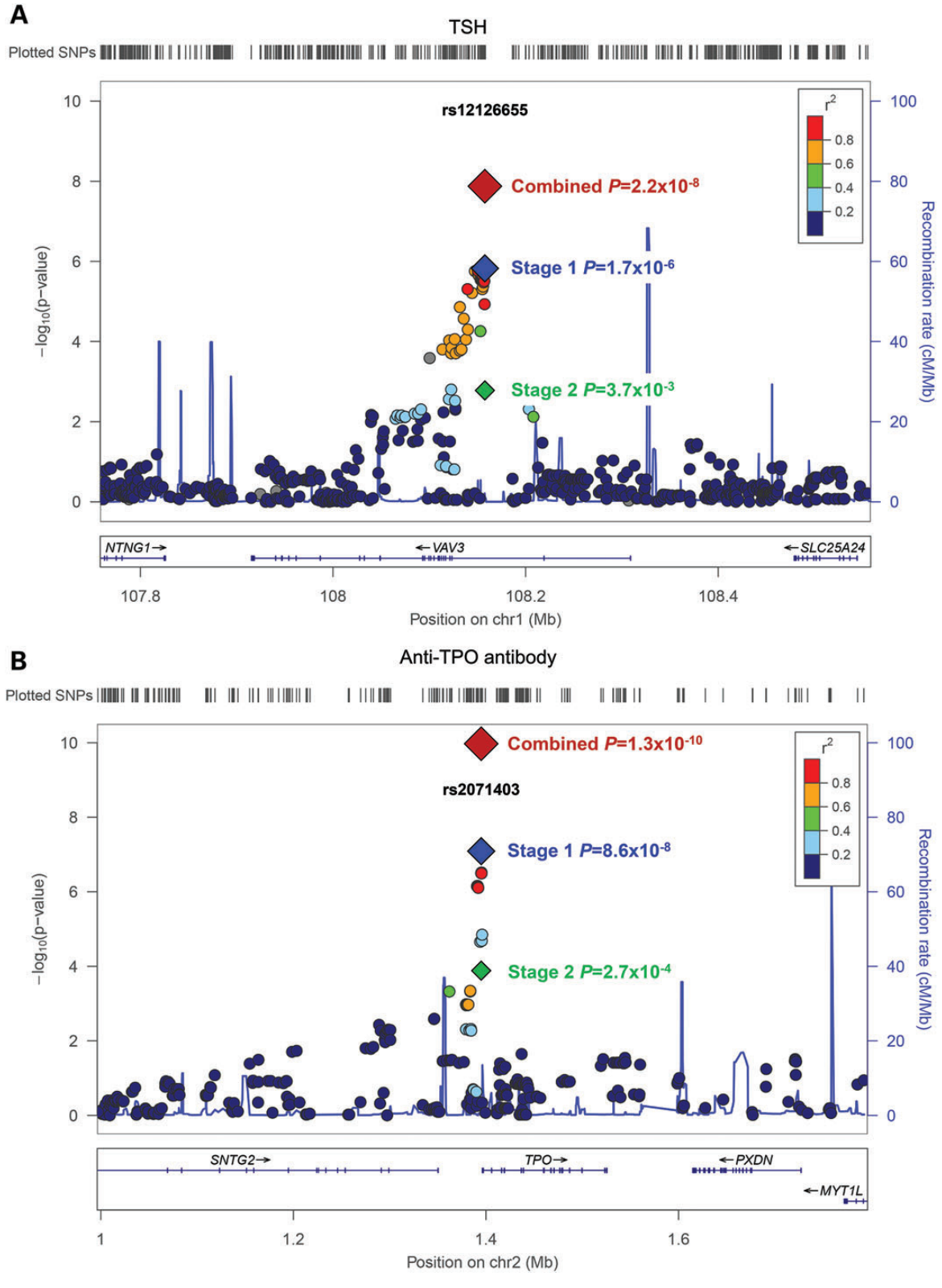

Figure 1. A regional association plot showing associations with TSH concentration (**A**) and anti-TPO antibody (**B**). The hash marks above the panel show the location of each variant. The *P*-values from the association testing are shown in the negative $\log_{10}$ scale on the *y*-axis. The blue diamond indicates the variant with the most significant association in the Stage 1 genome scan. The green and red diamonds represent the association results in Stage 2 and joint Stages 1 and 2 analyses, respectively. The variants in LD with the most significant SNPs are color-coded to represent their LD strength in HapMap CHB/JPT. The locations of genes, exons and their transcription direction are shown in the lower panel (derived from the Human Genome hg18 build).

and anti-TPO antibody positivity (rs733208 in *HLA-DPB2*, $P = 4.2 \times 10^{-7}$). We also limited our subjects to those who had normal TSH, and normal fT_4 concentrations, and were absent of anti-TPO antibody ($N = 2794$) and observed a similar association trend (Supplementary Material, Table S4).

Subgroup analysis according to sex or subclinical hypothyroidism

We investigated whether there was a sex-specific effect of the two variants that reached genome-wide significance (rs12126655 and rs2071403) (Table 3). Although the effect size of the rs12126655 variant was larger in females compared with males, the difference in effect size was not significant between the two groups. Regarding the rs2071403 variant, the effect size was also larger in females compared with males, and there was a marginal significance in difference of effect size ($P = 0.063$). We further performed a case–control analysis by comparing subgroups of euthyroid subjects ($N = 3056$) and those who had subclinical hypothyroidism ($N = 480$) (Supplementary Material, Table S5). The rs12126655 variant in *VAV3* was associated with subclinical hypothyroidism with a significance of $P < 0.05$ in both Stages 1 and 2 analyses, thus resulting in a joint Stages 1 and 2 significance of $P = 1.1 \times 10^{-3}$. The rs2071403 variant in *TPO* was not associated subclinical hypothyroidism ($P = 0.404$). The phenotypic variances explained by genome-wide SNPs in the Stage 1 participants were analysed with a polygenic mixed linear model using Genome-Wide Complex Trait Analysis (GCTA) (15). We estimated that the variance explained by common variants across the genome was 17.1% for TSH, 27.6% for fT4, and 15.1% for anti-TPO antibody positivity.

Pathway analysis

We performed pathway analysis using Database for Annotation, Visualization and Integrated Discovery (DAVID) with genes

Table 3. Sex-specific effect of rs1212665 and rs2071403 on TSH concentration and anti-TPO antibody positivity

					Male				Female			
Variant	Chr	Position (bp)	A1	Nearby gene	*N*	β/OR	SE	*P*	*N*	β/OR	SE	*P*
rs12126655	1	108 158 343	G	*VAV3*	1535	0.108	0.036	2.7×10^{-3}	1964	0.157	0.034	2.7×10^{-6}
rs2071403	2	1 396 251	A	*TPO*	1881	0.749	0.135	3.2×10^{-2}	2337	0.551	0.095	4.1×10^{-10}

	Joint male and female					
	N	β/OR	SE	*P*	I^2	Het *P*
rs12126655	3499	0.134	0.025	6.1×10^{-8}	0	0.322
rs2071403	4218	0.610	0.078	2.3×10^{-10}	71	0.063

Associations were tested with minor alleles by linear regression for TSH concentration and by logistic regression for anti-TPO antibody positivity. For TSH concentration, the effect size is shown as β and for anti-TPO antibody positivity as odds ratio (OR). The minor allele and its physical position are indexed to the positive strand of the NCBI build 36. The nearby gene is defined as the gene nearest to the variant within the boundary of the 50-kb distance. Chr, chromosome number; A1, minor allele; *N*, number of subjects; MAF, minor allele frequency; β, effect size of linear regression; SE, standard error; I^2, heterogeneity estimate; Het *P*, *P*-value for heterogeneity.

Table 4. Pathway analysis of top genes associated with anti-TPO antibody positivity (FDR by Benjamini–Hochberg <0.5)

Database code	Annotation term	No. of genes	%	*P*	Genes
Gene Ontology Biological Process Level 3					
GO:0048002	Antigen processing and presentation of peptide antigen	4	12.5	1.21×10^{-5}	*MICA, HLA-B, HLA-G, HLA-F*
GO:0006952	Defense response	4	12.5	7.67×10^{-2}	*MICA, TACR1, HLA-B, HLA-G*
GO:0009408	Response to heat	2	6.2	8.77×10^{-2}	*MICA, TACR1*
KEGG Pathway					
hsa05320	Autoimmune thyroid disease	5	15.6	1.80×10^{-6}	*TPO, HLA-DPB1, HLA-B, HLA-G, HLA-F*
hsa05330	Allograft rejection	4	12.5	3.78×10^{-5}	*HLA-DPB1, HLA-B, HLA-G, HLA-F*
hsa05332	Graft-versus-host disease	4	12.5	4.82×10^{-5}	*HLA-DPB1, HLA-B, HLA-G, HLA-F*
hsa04940	Type I diabetes mellitus	4	12.5	6.04×10^{-5}	*HLA-DPB1, HLA-B, HLA-G, HLA-F*
hsa05416	Viral myocarditis	4	12.5	2.92×10^{-4}	*HLA-DPB1, HLA-B, HLA-G, HLA-F*
hsa04612	Antigen processing and presentation	4	12.5	4.63×10^{-4}	*HLA-DPB1, HLA-B, HLA-G, HLA-F*
hsa04514	Cell adhesion molecules	4	12.5	1.80×10^{-3}	*HLA-DPB1, HLA-B, HLA-G, HLA-F*
hsa04650	Natural killer cell mediated cytotoxicity	3	9.4	2.66×10^{-2}	*MICA, HLA-B, HLA-G*
hsa04144	Endocytosis	3	9.4	4.84×10^{-2}	*HLA-B, HLA-G, HLA-F*

DAVID analysis for functional annotation was performed with GO, and KEGG database. The *P*-value is from the modified Fisher exact test provided from the DAVID system. The genes submitted as input were: *ASTN2*, *BCL2L11*, *C11orf41*, *C4orf35*, *C6orf10*, *CAPRIN2*, *COL11A2*, *CTNND2*, *HEATR1*, *HLA-B*, *HLA-DPB1*, *HLA-DPB2*, *HLA-F*, *HLA-G*, *IPO8*, *KCNIP1*, *LARGE*, *MICA*, *NELL2*, *PDCD10*, *PIGN*, *PKNOX2*, *POLE4*, *RGS9*, *RPS6KA2*, *RSPO2*, *SDK1*, *TACR1*, *TPO*, *TSH*, *UBD*, *WDR49* and *ZNF8*.

annotated by top listed variants (16,17). Using the functional annotation tool we found that 'Antigen processing and presentation of peptide antigen' was the most significantly enriched annotation term in Gene Onotology (GO) biological process database ($P = 1.2 \times 10^{-5}$) (18) and 'Autoimmune thyroid disease' was the most significantly enriched term in Kyoto Encyclopedia of Genes and Genomes (KEGG) database ($P = 1.8 \times 10^{-6}$) (19) regarding anti-TPO antibody (Table 4). However, we were not able to identify a significantly enriched GO term or KEGG pathway using the genes annotated from the TSH or fT_4 association results.

The expression of *TPO* in human thyroid tissue

Among the two variants that passed the threshold for the genome-wide significance, rs12126655 variant was located in intron of *VAV3* and it was not feasible to perform a functional analysis. Regarding the rs2071403 in the *TPO* gene, we

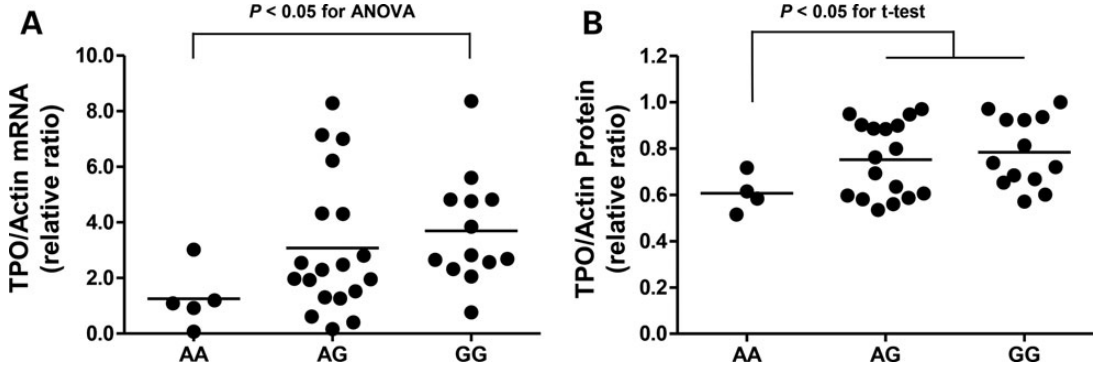

Figure 2. The relative expression of TPO mRNA (**A**) and protein (**B**) in human thyroid tissues according to the rs2071403 genotypes. There was a significant difference in TPO mRNA expression according to the three genotype groups [$P < 0.05$ for the analysis of variance (ANOVA)]. TPO protein expression was decreased in the AA group compared to the AG and GG groups ($P < 0.05$ for Student's *t*-test).

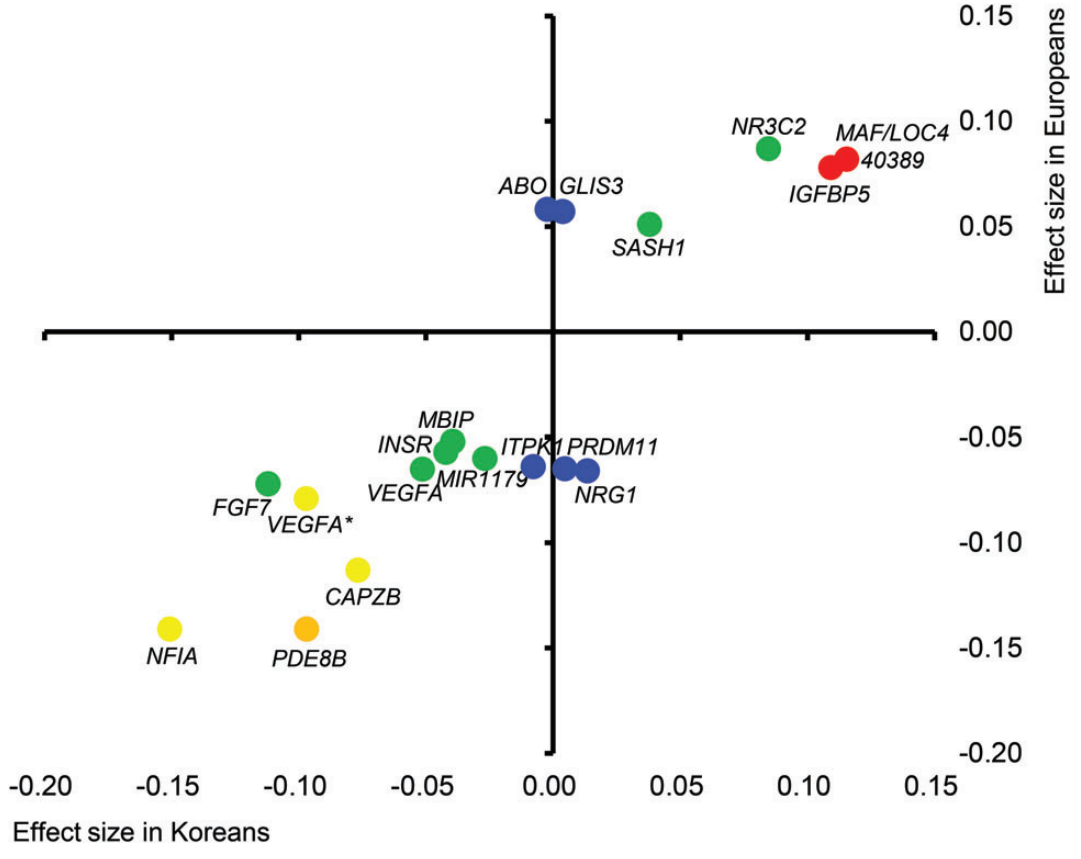

Figure 3. A comparison of variant effect size associated with TSH between Koreans and Europeans. For the variants associated with TSH concentrations in a recent European meta-GWAS (9), the effect sizes (β) for Koreans (*x*-axis) and Europeans (*y*-axis) are plotted with the corresponding Korean *P*-values. $P < 0.0001$, red; $0.0001 \leq P < 0.01$, orange; $0.01 \leq P < 0.10$, yellow; $0.10 \leq P < 0.50$, green; and $0.50 \leq P$, blue. The two *VEGFA* variants are distinguished by *VEGFA** for rs9472138 and *VEGFA* for rs11755845.

studied the putative functional role of this variant by comparing the expression level of *TPO* in human thyroid tissues according to the genotypes. Thyroid tissues were used from 37 participants who had thyroid tumours (benign or malignant) and underwent thyroidectomy. The participants who had the rs2071403A variant, which was associated with lower anti-TPO antibody positivity, had a significantly decreased TPO mRNA expression level in their thyroid tissue (Fig. 2A). However, the protein level was only decreased in participants with the AA genotype compared to those with the AG or GG genotype (Fig. 2B).

A comparison with the European GWAS results

The effect sizes (β) reported in the recent meta-analysis of the GWAS regarding TSH in Europeans (7) were compared with those from our Stage 1 genome scan (Fig. 3 and Supplementary Material, Table S6). For TSH concentration, 20 variants reported in Europeans were available for analyses in our study, and six variants showed significant associations with $P < 0.05$ in the identical direction of association. There was a significant positive correlation between effect sizes in the two populations (Pearson's correlation coefficient 0.864 and $P = 4.0 \times 10^{-6}$). We also investigated the variants that were confirmed to be associated with autoimmune thyroid disease (including Graves' disease and Hashimoto's thyroiditis) (Supplementary Material, Table S7) (11–13). Among the 15 variants investigated, three were nominally ($P < 0.05$) associated with plasma TSH concentration and three other variants were associated with anti-TPO antibody positivity ($P < 0.05$).

DISCUSSION

Using two-stage genome-wide association analyses, we replicated and confirmed that the rs12126655 variant in *VAV3* was associated with TSH concentration and observed a novel genome-wide significant association between the rs2071403 variant at the 5′ UTR of *TPO* and anti-TPO antibody positivity in a Korean population. We also identified two variants that had suggestive genome-wide significant associations ($P < 5.0 \times 10^{-7}$) with fT_4 concentration (rs7951105 in 11p12) and anti-TPO antibody positivity (rs733208 in *HLA-DPB2*). To our best knowledge, this is the first study in Asians to investigate genetic variants affecting TSH and fT_4 concentrations as a continuous trait at a genome-wide scale. In addition, this is the first study to identify a presumptive functional variant affecting anti-TPO antibody positivity with unequivocal significance.

The *VAV3* encodes for vav 3 guanine nucleotide exchange factor, which activates Rho and Rac GTPase. *VAV3* is involved in immune function, sympathetic hyperactivity and oncogenesis (20–22). The functional role of *VAV3* in TSH regulation is not fully understood. *VAV3* is widely expressed in several tissues, including peripheral lymphocytes, the spleen, brain and thyroid gland (23). In 27 758 Icelanders, the rs17020124 variant, which is in strong LD ($r^2 = 0.84$ in HapMap CHB/JPT) with rs12126655, was associated with TSH concentration with genome-wide significance ($P = 4.3 \times 10^{-17}$) (8). In another recent GWAS involving 39 248 Europeans, the rs4915077 variant in *VAV3* was associated with self-reported hypothyroidism ($P = 7.5 \times 10^{-10}$) (24). This variant was also in strong LD with rs12126655 ($r^2 = 0.86$ in HapMap CHB/JPT). The rs12126655 was associated with subclinical hypothyroidism in both our Stages 1 and 2 participants with nominal significance. These results indicate that variants in *VAV3* are important determinants of TSH concentration and affect thyroid dysfunction across different ethnicities.

A notable result of this study was the association between the rs2071403 variant at the 5′ UTR of *TPO* gene and anti-TPO antibody positivity. The *TPO* gene encodes for TPO and is highly expressed in human thyroid tissue. This gene plays a critical role in thyroid hormone synthesis by iodinating tyrosine residues in thyroglobulin and phenoxy-ether formation between iodinated tyrosine residues (25,26). Inactivating mutations in the *TPO* gene is known to cause an iodide organification defect that results in congenital hypothyroidism (27,28). Because the rs2071403 variant was located at the 5′ UTR of the *TPO* gene 75 bp proximal to the translation start site, we thought that it could influence the expression level of the *TPO* gene. We investigated the mRNA and protein expression levels in human thyroid tissue and observed that the expression levels are different according to the rs2071403 genotype. However, the G to A variation of rs2071403 does not appear to alter transcription factor-binding site (29), and a detailed molecular mechanism of how this variant affects expression levels should be further investigated. Currently, it is unclear whether the rs2071403 variant is merely a marker that is in LD with a nearby functional variant. It could be postulated that the rs2071403 variant is associated with a decreased expression of the *TPO* gene in thyroid tissues and thus results in a lower rate of anti-TPO antibody positivity. The variant was also marginally associated with decreased fT_4 concentration in our joint Stages 1 and 2 analyses ($\beta = -0.044$, $P = 0.071$). The decrease in fT_4 concentration might also be attributed to decreased *TPO* gene expression and altered hormonal synthesis in the thyroid tissue.

When we compared the TSH-associated variants reported in Europeans with our Stage 1 analysis, we observed a significant positive linear correlation of effect size between the two populations. Among the variants reported in Europeans, rs3813582 near *MAF/LOC440389* and rs13015993 near *IGFBP5* showed strong associations in our Stage 1 analysis, albeit not passing the significance threshold of the Stage 1 analysis ($P = 4.4 \times 10^{-5}$ and 2.9×10^{-5}). The most significantly associated genetic variant for TSH in Europeans was rs6885099, which is located in the intron of *PDE8B* (*phosphodiesterase 8B*). This variant also had a nominal association ($P = 5.9 \times 10^{-3}$) with TSH in our Stage 1 subjects. These results imply that the genetic variants affecting thyroid function may be similar between the two populations. It is not clear whether the difference in the prevalence of subclinical hypothyroidism between the US and the Korean population could be explained by genetic predisposition. Excessive iodine intake is associated with higher risk of subclinical hypothyroidism (30). As iodine intake in Korean population is amongst the highest except for the Japanese, the difference might be more likely associated with difference in iodine intake (31).

A major limitation of our study regards the relatively modest sample size for genome-wide association analyses. For the quantitative trait analyses, we had 87% power to detect a genome-wide significant association for variants with a minor allele frequency of 0.35 and an effect size of 0.15 in 3500 subjects. However, the power decreased as the effect size reduced to

0.14 and 0.13 by 75 and 60%, respectively. For the case–control analyses, we only had sufficient power to detect association results for variants with odds ratios of 1.6 or more, assuming a minor allele frequency of 0.35 and an anti-TPO antibody positive rate of 0.11 in 4200 subjects. Therefore, we might have missed a significant portion of true positive associations. Further replications and meta-analysis would be required to confirm our findings and to discover additional genetic variants regarding thyroid function and anti-TPO antibody positivity.

In conclusion, we performed a genome-wide association analysis on TSH and fT_4 concentrations and anti-TPO antibody positivity in Koreans. We confirmed that an intronic variant of *VAV3*, rs12126655, was associated with TSH concentration with genome-wide significance. We also identified a novel association between a variant at the 5′ UTR of the *TPO* gene and anti-TPO antibody positivity. This variant showed marginal sex-specific effects and was associated with TPO mRNA expression in human thyroid tissues. We hope these results can expand our knowledge of thyroid hormone synthesis, secretion, and autoimmunity and eventually provide a basis for personalized medicine in autoimmune thyroid disease.

MATERIALS AND METHODS

Study participants for the Stage 1 genome scan

Participants for the Stage 1 genome scan were recruited from the Ansung cohort based on Anusung-Si, GyeongGi-Do, Korea, as a component of the KoGES (32,33). The initial investigation began in 2001 with 5018 participants aged 40–69. Participants with a previous history of thyroid disease or on medications (thyroid hormone, anti-thyroid medications, steroid, estrogen and contraceptives) that could affect thyroid hormone concentration were excluded. The total number of subjects with plasma TSH and fT_4 concentration was 2789, and the number of subjects with anti-TPO antibody measurement was 3396. The baseline characteristics of the study subjects are shown in Table 1.

Study participants for the Stage 2 follow-up

Participants for the Stage 2 follow-up were from the KLoSHA cohort (34). The cohort was initiated in 2005 for 953 participants 65 years old or older who lived in SeongNam-Si, GyeongGi-Do, Korea. Participants with a previous history of thyroid disease or taking the above medications were also excluded. A total of 829 subjects had plasma TSH, fT_4 and anti-TPO antibody measurements. The institutional review board of the Biomedical Research Institute at Seoul National University Hospital approved the study protocol (H-1309-075-521), and written informed consent was obtained from each subject. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Measurements of TSH, fT_4 and anti-TPO antibody

For both Stages 1 and 2 participants, the plasma TSH concentrations were measured using a radioimmunoassay (RIA) kit from CIS Bio International S.A., Gif-sur-Yvette, France (normal range 0.4–4.1 μIU/l), and the plasma fT_4 concentrations were measured using an RIA kit from DiaSorin S.p.A., Saluggia, Italy (normal range 0.7–1.8 ng/dl). Plasma anti-TPO antibody concentrations were measured with an RIA kit from RSR Ltd, Pentwyn, UK (normal range <0.3 μIU/l) in Stage 1 participants and with an RIA kit from BRAHMS, Hennigsdorf, UK (normal range <25 μIU/l) in Stage 2 subjects. Participants with an anti-TPO antibody concentration above the normal range were classified as anti-TPO antibody positive cases and the remaining participants were controls. Because the variables showed a significant deviation from a Gaussian distribution, each of the TSH and fT_4 concentrations was inverse normal transformed before the analyses.

Genotyping and imputation

We extracted genomic DNA from the participants' peripheral leukocytes. The Stage 1 genome scan was performed using an Affymetrix Genome-Wide Human SNP Array 5.0. Only unrelated subjects with genotype missingness $<5\%$ were included in the analysis. Markers with significant deviations from the Hardy–Weinberg equilibrium ($P < 1.0 \times 10^{-6}$), a genotype call rate <0.95 and minor allele frequency <0.01 were excluded. A total of 351 669 SNPs were directly genotyped and were available for analyses. For the Stage 2 follow-up genotyping, we used the TaqMan assay from Applied Biosystems, Carlsbad, CA, USA. Overall, the TaqMan genotyping success rate was 99.5%, and the concordance rate based on 15 blind duplicate comparisons was 100% in the Stage 2 study. Imputation was performed using IMPUTE software (https://mathgen.stats.ox.ac.uk/impute) (35). The HapMap phased genotype information of CHB and JPT (build 36 release 22) was used as a reference (36). Imputed SNPs with high genotype information content (info >0.5) were used. The identical quality control criteria were applied for the imputed SNPs as in the genotyped SNPs. After filtering, a total of 1 418 709 SNPs were available for the analyses.

Statistical analyses

Most of the association testing was performed using PLINK version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) (37). For plasma TSH and fT_4 concentrations, a linear regression assuming an additive genotypic model was used. For the plasma anti-TPO antibody concentration, a logistic regression (anti-TPO antibody positive versus negative subjects) was used. Age and sex were used as covariates. The full result of the Stage 1 genome scan can be downloaded in our webpage (http://bri.snuh.org/bench/_/notice/5250/view.do). Variants that passed the significance threshold ($P < 1.0 \times 10^{-5}$) in the Stage 1 genome scan were further genotyped in Stage 2. The significance threshold for the Stage 2 follow-up was $P < 0.05$. A joint analysis of Stages 1 and 2 was performed with METAL (http://www.sph.umich.edu/csg/abecasis/Metal/) using an inverse-variance meta-analysis method assuming fixed effects (38). Genome-wide significance was considered as $P < 5.0 \times 10^{-8}$. To account for possible cryptic relatedness and population stratification in the Stage 1 genome scan, we performed additional analyses using EMMAX (http://www.sph.umich.edu/csg/kang/emmax/) (14). To identify sex-specific effects, the analyses were performed separately for each sex, and we tested heterogeneity between effect sizes ($P < 0.05$ to be significant). The estimated proportion of phenotypic variance explained by the

genome-wide SNPs was calculated using GCTA software (http://www.complextraitgenomics.com/software/gcta) (37). The effect sizes of variants associated with TSH in the recent European meta-GWAS (27) and our Stage 1 genome scan were compared. For the pathway analysis, we used functional annotation tool in the DAVID system (http://david.abcc.ncifcrf.gov/) (17,18). We searched the GO (18) and KEGG database (19). The genes that are annotated by the variants that had false discovery rate (FDR) of <0.5 by Benjamini–Hochberg procedure in our Stage 1 genome scan were selected for input. The corresponding cut-off *P*-values for the association with TSH, fT_4 and anti-TPO antibody were 1.3×10^{-5}, 1.7×10^{-4} and 1.2×10^{-4}, respectively. The *P*–value of <0.01 for the modified Fisher exact test provided from the DAVID system was considered to be significant.

Thyroid tissue preparation

Thyroid tissue was prepared from 37 participants who had thyroid tumours and underwent thyroidectomy from February 2012 to March 2013. The normal thyroid tissue in a $1 \times 1 \times 1$ cm^3 volume was dissected from the contralateral lobe without a tumour and was immediately stored in a liquid nitrogen tank. Thyroid tissues were collected for the biobank program of the Department of Surgery, Seoul National University Hospital. The biobank study protocol was approved by the institutional review board of the Biomedical Research Institute at Seoul National University Hospital (H-0809-097-258). Each participant provided written informed consent for the study.

RNA extraction and real-time PCR analysis

Total RNA was isolated from normal thyroid tissues using TRizol reagent (Ambion, Foster City, CA, USA), and M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) was used for cDNA synthesis. Real-time PCR was performed using SYBR-master mix (Takara, Otsu, Shiga, Japan) and an ABI 7500 real-time PCR system (Applied Biosystems, Forster City, CA, USA). Each sample was analysed in duplicate. The following primer sequences were used for the real-time PCR: for TPO (189 bp), forward primer 5′-CGGGTCATCTGTGACAACAC-3′, reverse primer 5′-GT GCACAAAGTCCCCATTCT-3′; and for beta-actin (205 bp), forward primer 5′-TGACGTGGACATCCGC AAAG-3′, reverse primer 5′-CTGGAAGGTGGACAGCGAGG-3′.

Western blot analysis

Normal thyroid tissues were lysated with 20 mM Tris (pH 7.4), 5 mM EDTA and 1% NP-40, supplemented with a protease inhibitor mixture in 1 : 100 dilution (Sigma–Aldrich, St Louis, MO, USA), separated by SDS–PAGE and transferred to a Hybond ECL nitrocellulose membrane (Amersham, Piscataway, NJ, USA). To assess TPO expression, the nitrocellulose membranes were incubated with an anti-TPO antibody (Abcam, Cambridge, MA, USA) at a 1 : 3000 dilution overnight at 4°C, and then subsequently incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Cell Signaling, Boston, MA, USA) at a 1 : 5000 dilution for 1 h at room temperature. The signals were detected with the ECL Western Blotting Analysis System (Thermo Fisher Scientific, Waltham, MA, USA). For a loading control, β-actin immunoreactivity was detected with a monoclonal anti-β-actin antibody (Sigma–Aldrich, St Louis, MO, USA) at a 1 : 5000 dilution.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENT

We thank Dr Jung Hun Ohn for his advice and comments on performing the pathway analysis. This study was supported by a grant from the Korean Health Technology R&D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (grant no. A092077).

Conflict of Interest statement. None declared.

FUNDING

This work was supported by a grant from the National Project for Personalized Genomic Medicine, Ministry for Health and Welfare (grant no. A111218-GM09); the National Genome Research Institute, Korean Center for Disease Control and Prevention (contract #2001-347-6111-221, 2002-347-6111-221, 2003-347-6111-221, 2004-E71001-00, 2005-E71001-00, 2006-E71005-00, 2006-E71006-00, 2007-E71001-00, 2007-E71003-00, 2008-E71001-00, 2008-E71005-00, 2009-E71002-00, 2009-E71007-00, 2010-E71001-00, 2010-E71004-00, 2011-E71004-00, 2011-E71008-00, 2012-E71008-00, 2012-E71005-00, and grant no. 4845-301); and an intramural grant from the Korea National Institute of Health (grant no. 2012-N73002-00), Republic of Korea.

REFERENCES

1. Bianco, A.C. and Kim, B.W. (2006) Deiodinases: implications of the local control of thyroid hormone action. *J. Clin. Invest.*, **116**, 2571–2579.
2. Visser, W.E., Friesema, E.C. and Visser, T.J. (2011) Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol. Endocrinol.*, **25**, 1–14.
3. Andersen, S., Pedersen, K.M., Bruun, N.H. and Laurberg, P. (2002) Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *J. Clin. Endocrinol. Metab.*, **87**, 1068–1072.
4. Dayan, C.M. and Panicker, V. (2009) Novel insights into thyroid hormones from the study of common genetic variation. *Nat. Rev. Endocrinol.*, **5**, 211–218.
5. Arnaud-Lopez, L., Usala, G., Ceresini, G., Mitchell, B.D., Pilia, M.G., Piras, M.G., Sestu, N., Maschio, A., Busonero, F., Albai, G. *et al.* (2008) Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. *Am. J. Hum. Genet.*, **82**, 1270–1280.
6. Panicker, V., Wilson, S.G., Walsh, J.P., Richards, J.B., Brown, S.J., Beilby, J.P., Bremner, A.P., Surdulescu, G.L., Qweitin, E., Gillham-Nasenya, I. *et al.* (2010) A locus on chromosome 1p36 is associated with thyrotropin and thyroid function as identified by genome-wide association study. *Am. J. Hum. Genet.*, **87**, 430–435.
7. Lowe, J.K., Maller, J.B., Pe'er, I., Neale, B.M., Salit, J., Kenny, E.E., Shea, J.L., Burkhardt, R., Smith, J.G., Ji, W. *et al.* (2009) Genome-wide association studies in an isolated founder population from the Pacific Island of Kosrae. *PLoS Genet.*, **5**, e1000365.
8. Gudmundsson, J., Sulem, P., Gudbjartsson, D.F., Jonasson, J.G., Masson, G., He, H., Jonasdottir, A., Sigurdsson, A., Stacey, S.N., Johannsdottir, H. *et al.*

(2012) Discovery of common variants associated with low TSH levels and thyroid cancer risk. *Nat. Genet.*, **44**, 319–322.
9. Porcu, E., Medici, M., Pistis, G., Volpato, C.B., Wilson, S.G., Cappola, A.R., Bos, S.D., Deelen, J., den Heijer, M., Freathy, R.M. *et al.* (2013) A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. *PLoS Genet.*, **9**, e1003266.
10. Simmonds, M.J. (2013) GWAS in autoimmune thyroid disease: redefining our understanding of pathogenesis. *Nat. Rev. Endocrinol.*, **9**, 277–287.
11. Chu, X., Pan, C.M., Zhao, S.X., Liang, J., Gao, G.Q., Zhang, X.M., Yuan, G.Y., Li, C.G., Xue, L.Q., Shen, M. *et al.* (2011) A genome-wide association study identifies two new risk loci for Graves' disease. *Nat. Genet.*, **43**, 897–901.
12. Cooper, J.D., Simmonds, M.J., Walker, N.M., Burren, O., Brand, O.J., Guo, H., Wallace, C., Stevens, H., Coleman, G., Franklyn, J.A. *et al.* (2012) Seven newly identified loci for autoimmune thyroid disease. *Hum. Mol. Genet.*, **21**, 5202–5208.
13. Zhao, S.X., Xue, L.Q., Liu, W., Gu, Z.H., Pan, C.M., Yang, S.Y., Zhan, M., Wang, H.N., Liang, J., Gao, G.Q. *et al.* (2013) Robust evidence for five new Graves' disease risk loci from a staged genome-wide association analysis. *Hum. Mol. Genet.*, **22**, 3347–3362.
14. Kang, H.M., Sul, J.H., Service, S.K., Zaitlen, N.A., Kong, S.Y., Freimer, N.B., Sabatti, C. and Eskin, E. (2010) Variance component model to account for sample structure in genome-wide association studies. *Nat. Genet.*, **42**, 348–354.
15. Yang, J., Lee, S.H., Goddard, M.E. and Visscher, P.M. (2011) GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.*, **88**, 76–82.
16. Huang da, W., Sherman, B.T. and Lempicki, R.A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.*, **4**, 44–57.
17. Huang da, W., Sherman, B.T. and Lempicki, R.A. (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.*, **37**, 1–13.
18. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T. *et al.* (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.*, **25**, 25–29.
19. Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y. and Hattori, M. (2004) The KEGG resource for deciphering the genome. *Nucleic Acids Res.*, **32**, D277–D280.
20. Dumont, C., Corsoni-Tadrzak, A., Ruf, S., de Boer, J., Williams, A., Turner, M., Kioussis, D. and Tybulewicz, V.L. (2009) Rac GTPases play critical roles in early T-cell development. *Blood*, **113**, 3990–3998.
21. Sauzeau, V., Sevilla, M.A., Rivas-Elena, J.V., de Alava, E., Montero, M.J., Lopez-Novoa, J.M. and Bustelo, X.R. (2006) Vav3 proto-oncogene deficiency leads to sympathetic hyperactivity and cardiovascular dysfunction. *Nat. Med.*, **12**, 841–845.
22. Chang, K.H., Sanchez-Aguilera, A., Shen, S., Sengupta, A., Madhu, M.N., Ficker, A.M., Dunn, S.K., Kuenzi, A.M., Arnett, J.L., Santho, R.A. *et al.* (2012) Vav3 collaborates with p190-BCR-ABL in lymphoid progenitor leukemogenesis, proliferation, and survival. *Blood*, **120**, 800–811.
23. Movilla, N. and Bustelo, X.R. (1999) Biological and regulatory properties of Vav-3, a new member of the Vav family of oncoproteins. *Mol. Cell. Biol.*, **19**, 7870–7885.
24. Eriksson, N., Tung, J.Y., Kiefer, A.K., Hinds, D.A., Francke, U., Mountain, J.L. and Do, C.B. (2012) Novel associations for hypothyroidism include known autoimmune risk loci. *PloS ONE*, **7**, e34442.
25. Taurog, A., Dorris, M.L. and Doerge, D.R. (1996) Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. *Arch. Biochem. Biophys.*, **330**, 24–32.
26. Nunez, J. (1984) Thyroid hormones: mechanism of phenoxy ether formation. *Methods Enzymol.*, **107**, 476–488.
27. Bikker, H., Vulsma, T., Baas, F. and de Vijlder, J.J. (1995) Identification of five novel inactivating mutations in the human thyroid peroxidase gene by denaturing gradient gel electrophoresis. *Hum. Mutat.*, **6**, 9–16.
28. Park, S.M. and Chatterjee, V.K. (2005) Genetics of congenital hypothyroidism. *J. Med. Genet.*, **42**, 379–389.
29. Heinemeyer, T., Wingender, E., Reuter, I., Hermjakob, H., Kel, A.E., Kel, O.V., Ignatieva, E.V., Ananko, E.A., Podkolodnaya, O.A., Kolpakov, F.A. *et al.* (1998) Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. *Nucleic Acids Res.*, **26**, 362–367.
30. Teng, W., Shan, Z., Teng, X., Guan, H., Li, Y., Teng, D., Jin, Y., Yu, X., Fan, C., Chong, W. *et al.* (2006) Effect of iodine intake on thyroid diseases in China. *N. Engl. J. Med.*, **354**, 2783–2793.
31. Kim, J.Y., Moon, S.J., Kim, K.R., Sohn, C.Y. and Oh, J.J. (1998) Dietary iodine intake and urinary iodine excretion in normal Korean adults. *Yonsei Med. J.*, **39**, 355–362.
32. Cho, Y.S., Go, M.J., Kim, Y.J., Heo, J.Y., Oh, J.H., Ban, H.J., Yoon, D., Lee, M.H., Kim, D.J., Park, M. *et al.* (2009) A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat. Genet.*, **41**, 527–534.
33. Hong, K.W., Kim, S.S. and Kim, Y. (2013) Genome-wide association study of orthostatic hypotension and supine-standing blood pressure changes in two Korean populations. *Genomics Inform.*, **11**, 129–134.
34. Moon, J.H., Park, Y.J., Kim, T.H., Han, J.W., Choi, S.H., Lim, S., Park, D.J., Kim, K.W. and Jang, H.C. (2013) Lower-but-normal serum TSH level is associated with the development or progression of cognitive impairment including mild cognitive impairment and dementia in elderly: a result from Korean Longitudinal Study on Health and Aging (KLoSHA). *J. Clin. Endocrinol. Metab.*, **99**, 424–432.
35. Marchini, J., Howie, B., Myers, S., McVean, G. and Donnelly, P. (2007) A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.*, **39**, 906–913.
36. 2003) The International HapMap Project. *Nature*, **426**, 789–796.
37. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J. *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.*, **81**, 559–575.
38. Willer, C.J., Li, Y. and Abecasis, G.R. (2010) METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*, **26**, 2190–2191.