EnM
ENDOCRINOLOGY AND METABOLISM

Review Article

Endocrinol Metab 2018;33:175-184
https://doi.org/10.3803/EnM.2018.33.2.175
pISSN 2093-596X · eISSN 2093-5978

Genome-Wide Association Studies of Autoimmune Thyroid Diseases, Thyroid Function, and Thyroid Cancer

Yul Hwangbo[1], Young Joo Park[2]

[1]Center for Thyroid Cancer, National Cancer Center, Goyang; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea

Thyroid diseases, including autoimmune thyroid diseases and thyroid cancer, are known to have high heritability. Family and twin studies have indicated that genetics plays a major role in the development of thyroid diseases. Thyroid function, represented by thyroid stimulating hormone (TSH) and free thyroxine (T4), is also known to be partly genetically determined. Before the era of genome-wide association studies (GWAS), the ability to identify genes responsible for susceptibility to thyroid disease was limited. Over the past decade, GWAS have been used to identify genes involved in many complex diseases, including various phenotypes of the thyroid gland. In GWAS of autoimmune thyroid diseases, many susceptibility loci associated with autoimmunity (human leukocyte antigen [*HLA*], protein tyrosine phosphatase, non-receptor type 22 [*PTPN22*], cytotoxic T-lymphocyte associated protein 4 [*CTLA4*], and interleukin 2 receptor subunit alpha [*IL2RA*]) or thyroid-specific genes (thyroid stimulating hormone receptor [*TSHR*] and forkhead box E1 [*FOXE1*]) have been identified. Regarding thyroid function, many susceptibility loci for levels of TSH and free T4 have been identified through genome-wide analyses. In GWAS of differentiated thyroid cancer, associations at *FOXE1*, MAP3K12 binding inhibitory protein 1 (*MBIP*)-NK2 homeobox 1 (*NKX2-1*), disrupted in renal carcinoma 3 (*DIRC3*), neuregulin 1 (*NRG1*), and pecanex-like 2 (*PCNXL2*) have been commonly identified in people of European and Korean ancestry, and many other susceptibility loci have been found in specific populations. Through GWAS of various thyroid-related phenotypes, many susceptibility loci have been found, providing insights into the pathogenesis of thyroid diseases and disease co-clustering within families and individuals.

Keywords: Genome-wide association study; Graves disease; Hashimoto disease; Thyroid neoplasms; Thyroid function

INTRODUCTION

Most thyroid diseases, including autoimmune thyroiditis and thyroid cancer, have been recognized to have high heritability [1,2]. In twin studies, a high concordance rate for Graves' disease (GD) in monozygotic twins was reported, in the range of 50% to 70%, compared with 3% to 25% in dizygotic twins [1,3]. A study of autoimmune hypothyroidism likewise showed a 55% concordance in monozygotic twins [4]. Familial clustering of autoimmune thyroid disease has been consistently reported [5-7]. Hemminki et al. [7] showed that the familial standardized incidence ratios for GD were 4.49 for individuals with an affected parent, 5.04 for those whose singleton sibling was affected, 310 when two or more siblings were affected, and 16.45 in twins. For Hashimoto's thyroiditis (HT), the sibling risk ratio was 28 based on data from the National Health and Nutrition

Received: 3 May 2018, **Revised**: 8 May 2018, **Accepted:** 14 May 2018
Corresponding author: Young Joo Park
Department of Internal Medicine, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-4183, **Fax:** +82-2-764-2199, **E-mail:** yjparkmd@snu.ac.kr

Examination Survey III [8], and a similar risk was confirmed in data from Germany [5]. These pieces of evidence suggest the existence of a genetic predisposition to autoimmune thyroid diseases.

Thyroid function, including levels of thyroid hormone and thyroid stimulating hormone (TSH), is regulated within a narrow range in individuals, although the inter-individual variability is large [9]. This suggests that every individual has his or her own set point of thyroid function [10]. About 40% to 60% of variation in thyroid function has been estimated to be determined by genetic factors [10-12]. Thyroid cancers also show a high degree of heritability, with genetic factors accounting for more than 50% of the causes of thyroid cancer [2]. Except for medullary thyroid cancer, which is well known to be caused by germline or somatic mutations, the prevalence of familial differentiated thyroid cancer (DTC) accounted for 2.5% to 11.3% cases of DTC [13-17]. Only 5% of cases of nonmedullary familial DTC were reported to be of the syndromic form, which is accompanied by well-known germline mutations, including Cowden syndrome, familial adenomatous polyposis, Gardner syndrome, Carney complex type 1, Werner syndrome, and DICER1 syndrome [18]. Thus, the majority of cases of familial DTC were found not to be caused by germline mutations, despite its pattern of genetic inheritance.

Thus, genetics plays a prominent role in most thyroid-related phenotypes. Research into the genes responsible for thyroid disease has identified several candidates [19]. However, candidate gene studies have been controversial and have shown very few reproducible findings. Panicker [19] published a thorough review of genetic studies of thyroid function and autoimmune thyroid diseases conducted through 2010. In the last decade, genome-wide association studies (GWAS) have been extensively used to identify genes involved in complex diseases [20]. GWAS have facilitated the screening of a large proportion of the genome and discovered a variety of susceptibility genes. GWAS have been widely applied in autoimmune thyroid diseases, thyroid function, and thyroid cancer, and have identified susceptibility genes for thyroid-related phenotypes. Herein, we comprehensively review the wide range of discoveries from GWAS conducted in Western and Asian populations regarding autoimmune diseases, thyroid function, and thyroid cancer.

GWAS FOR AUTOIMMUNE THYROID DISEASES

Several candidate gene studies identified putative susceptibility variants for GD, but only the human leukocyte antigen (*HLA*) locus and the cytotoxic T-lymphocyte associated protein 4 (*CTLA4*), thyroid stimulating hormone receptor (*TSHR*), and protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*) loci were confirmed in subsequent replication studies [21-25]. The first genome-wide analysis using 14,436 nonsynonymous single-nucleotide polymorphisms (SNPs) for GD was performed by the Wellcome Trust Case Control Consortium, and showed that three loci (*HLA*, *TSHR*, and Fc receptor like 3 [*FCRL3*]) were associated with GD [26]. A subsequent GWAS with >500,000 SNPs confirmed previously reported loci and identified a novel region of susceptibility loci at 6q27 (the ribonuclease T2 [*RNASET2*]-FGFR1 oncogene partner [*FGFR1OP*]-CCR6) and an intergenic region at 4p14 (GDCG4p14) [27]. Several GWAS of autoimmune thyroid diseases (GD, HT, and positivity of anti-thyroid peroxidase [TPO] antibody or anti-thyroglobulin [Tg] antibody) and hypothyroidism have further identified susceptibility loci (Table 1) [26-36]. Since GWAS of HT have been performed for a variety of phenotypes including self-reported hypothyroidism, biochemical hypothyroidism with positive antibodies, antibody positivity, and level of antibodies, caution is needed when interpreting the results. Several types of hypothyroidism might not have an autoimmune etiology, and autoimmunity does not necessarily lead to hypothyroidism. Thus, careful consideration regarding the phenotype is required when interpreting the biological mechanisms of the associated genes identified through GWAS of autoimmune thyroid diseases.

A heterogeneity analysis between GD and HT showed that GD and HT share several susceptibility loci (*HLA*, *PTPN22*, and *CTLA4*), while an association with *TSHR* was exclusively seen in GD patients. The majority of genes associated with autoimmune thyroid disease are thought to play a major role in autoimmune processes, including disrupted T-cell regulation and peripheral immune tolerance [37]. Variants in thyroid-specific loci, including *TSHR* and forkhead box E1 (*FOXE1*), could affect the immune recognition of autoantigens and antibody generation [37].

GWAS OF THYROID FUNCTION

Thyroid function, including levels of free thyroxine (T4) and TSH, is highly heritable even in euthyroid subjects. A large meta-analysis of GWAS of serum levels of TSH and free T4, in 26,420 and 17,520 euthyroid European individuals, respectively, was performed, identifying many susceptibility loci for levels of TSH (phosphodiesterase 8B [*PDE8B*], phosphodiesterase 10A

Table 1. Susceptibility Loci for Autoimmune Thyroid Disease Detected by Genome-Wide Association Studies

Phenotypes	Locus	Gene	Protein function	Population	Reference
GD, HT	1p13	*PTPN22*	Involvement in T-cell signaling	UK, USA	[28,30]
GD	10p15.1	*IL2RA*	Encoding CD25	UK	[28]
GD, HT	2q33.2	*CTLA4*	Inhibition of T-cell signaling	UK, Chinese Han, USA	[27,28,30,31]
GD	1q23.1	*FCRL3*	Regulation of B-cell signaling	UK, Chinese Han	[26-28,31]
GD, HT	6p21	HLA class I region	Endogenous antigen presentation for recognition by CD8+ T-cells	UK, Chinese Han, USA	[26,27,30,31]
GD, HT	6p21	HLA class II region	Exogenous antigen presentation for recognition by CD4+ T-helper cells	UK, Chinese Han, USA	[26,27,30,31]
GD	14q31.1	*TSHR*	Autoantigenic target in GD	UK, Chinese Han	[26-28,31]
GD, HT	6q27	*RNASET2-FGFR1OP*	A fusion partner for FGFR1 in the t(6;8) (q27;p11) translocations	UK, Chinese Han	[27,28,31]
GD	4p14	*CHRNA9-RHOH*	Negative regulator of hematopoietic cell growth and survival	Chinese Han	[27,31]
GD	1p36.32	*MMEL1*	Role in pain perception, arterial pressure regulation, phosphate metabolism, and homeostasis	UK	[28]
GD	12q12	*PRICKLE1*	Negative regulator of the Wnt/β-catenin signaling pathway	UK	[28]
GD	16p11.2	*ITGAM*	Role in leukocyte adhesion to platelets and fibrinogen	UK	[28]
GD	Xq21.1	*GPR174-ITM2A*	Thymocyte selection and T-cell activation	Chinese Han	[31,32]
GD	22q12.3–13.1	*C1QTNF6-RAC2*	Role in elicitation of immune responses and the induction of peripheral immune tolerance	Chinese Han	[31]
GD	1q23.2	*SLAMF6*	Coreceptor in the process of NK cell activation	Chinese Han	[31]
GD	9q34.2	*ABO*	Determination of ABO blood group	Chinese Han	[31]
GD	14q32.	*C14orf64*	Long intergenic non-protein coding RNA 1550 (LINC01550)	Chinese Han	[31]
GD	8q24.22	*TG*	Encoding thyroglobulin	Chinese Han	[31]
HT	9q22.33	*FOXE1*	Encoding TTF-2, role in thyroid morphogenesis	USA	[29,30]
HT	12q24.12	*SH2B3*	Negative regulator of cytokine signaling	USA	[30]
HT	1p13.3	*VAV3*	Role in actin cytoskeletal rearrangements and transcriptional alterations	US, Japan	[30,36]
HT	1p36.13	*CAPZB*	Role in regulating actin filament dynamics	USA	[30]
HT	5q13.3	*PDE8B*	Role in hydrolysis of the second messenger cAMP	USA	[30]
GD, HT	2p25.1	*TRIB2*	Role in apoptosis of hematopoietic cells	UK	[28]
GD, HT	3q27.3	*LPP*	Involvement in cell-cell adhesion and cell motility	UK	[28]
GD, HT, TPOAb	6q15	*BACH2*	Role in coordinating transcription activation and repression by MAFK	UK, European[a]	[28,34]
GD, HT	11q21	*FAM76B*	Role in NEDD8-specific protease activity	UK	[28]
TPOAb	2p25.3	*TPO*	Encoding thyroid peroxidase	European, Korea	[33,34]
TPOAb	12q24.12	*ATXN2*	Role in Akt signaling and checkpoint regulation.	European	[34]
TPOAb	1p13.2	*MAGI3*	Role in Sertoli-Sertoli cell junction dynamics and Ras signaling pathway	European	[34]
TPOAb	3q21.1	*KALRN*	Role in p75 NTR-mediated signaling and EPH-ephrin signaling	European	[34]
TPOAb	9q31.1	*GRIN3A*	Role in circadian entrainment	Croatia	[35]
TgAb	6q27	*DLL1*	Role in mediating cell fate decisions during hematopoiesis	Croatia	[35]

GD, Graves' disease; HT, Hashimoto's thyroiditis or hypothyroidism; *PTPN22*, protein tyrosine phosphatase, non-receptor type 22; *IL2RA*, interleukin 2 receptor subunit alpha; *CTLA4*, cytotoxic T-lymphocyte associated protein 4; *FCRL3*, Fc receptor like 3; HLA, human leukocyte antigen; *TSHR*, thyroid stimulating hormone receptor; *RNASET2*, ribonuclease T2; *FGFR1OP*, FGFR1 oncogene partner; FGFR1, fibroblast growth factor receptor 1; *CHRNA9*, cholinergic receptor nicotinic alpha 9 subunit; *RHOH*, ras homolog family member H; *MMEL1*, membrane metalloendopeptidase like 1; *PRICKLE1*, prickle planar cell polarity protein 1; *ITGAM*, integrin subunit alpha M; *GPR174*, G protein-coupled receptor 174; *ITM2A*, integral membrane protein 2A; *C1QTNF6*, C1q and TNF related 6; *RAC2*, Rac family small GTPase 2; *SLAMF6*, SLAM family member 6; NK, natural killer; *TG*, anti-thyroglobulin; *FOXE1*, forkhead box E1; TTF-2, thyroid transcription factor-2; *SH2B3*, SH2B adaptor protein 3; *VAV3*, vav guanine nucleotide exchange factor 3; *CAPZB*, capping actin protein of muscle Z-line subunit beta; *PDE8B*, phosphodiesterase 8B; cAMP, cyclic adenosine monophosphate; *TRIB2*, tribbles pseudokinase 2; *LPP*, LIM domain containing preferred translocation partner in lipoma; TPOAb, anti-thyroid peroxidase antibody; *BACH2*, BTB domain and CNC homolog 2; MAFK, MAF bZIP transcription factor K; *FAM76B*, family with sequence similarity 76 member B; NEDD8, neural precursor cell expressed, developmentally down-regulated 8; *TPO*, anti-thyroid peroxidase; *ATXN2*, ataxin 2; *MAGI3*, membrane associated guanylate kinase, WW and PDZ domain containing 3; *KALRN*, kalirin Rho-GEF kinase; NTR, neurotrophin receptor; *GRIN3A*, glutamate ionotropic receptor NMDA type subunit 3A; TgAb, anti-thyroglobulin antibody; *DLL1*, delta like canonical Notch ligand 1.

[a]European refers to European ancestry from various countries.

[*PDE10A*], capping actin protein of muscle Z-line subunit beta [*CAPZB*], *MAP*, vascular endothelial growth factor A [*VEGFA*], nuclear receptor subfamily 3 group C member 2 [*NR3C2*], insulin like growth factor binding protein 5 [*IGFBP5*], SRY-box 9 [*SOX9*], nuclear factor I A [*NFIA*], fibroblast growth factor 7 [*FGF7*], PR/SET domain 11 [*PRDM11*], microRNA 1179 [*MIR1179*], insulin receptor [*INSR*], *ABO*, inositol-tetrakisphosphate 1-kinase [*ITPK1*], neuregulin 1 [*NRG1*], MAP3K12 binding inhibitory protein 1 [*MBIP*], SAM and SH3 domain containing 1 [*SASH1*], and GLIS family zinc finger 3 [*GLIS3*]) and levels of free T4 (iodothyronine deiodinase 1 [*DIO1*], LIM homeobox 3 [*LHX3*], *FOXE1*, aminoadipate aminotransferase [*AADAT*], lysophosphatidylcholine acyltransferase 2 [*LPCAT2*]/calpain small subunit 2 [*CAPNS2*], neuropilin and tolloid like 1 [*NETO1*]/F-box protein 15 [*FBXO15*]) [38]. A GWAS of TSH levels was also conducted in 1,346 Chinese Han individuals [39]. Zhan et al. [39] confirmed previously reported TSH susceptibility loci near *FOXE1* and *CAPZB* and identified novel variants in XK related 4 (*XKR4*). Whole-genome sequence-based analysis was performed to examine the genetic architecture for levels of free T4 and TSH, and further identified novel variants on synapsin II (*SYN2*), *PDE8B*, and beta-1,4-galactosyltransferase 6 (*B4GALT6*) [40]. They also found a rare functional variant (minor allele frequency=0.4%) in the transthyretin (*TTR*) gene, which is located near *B4GALT6*. This study showed that common variants explained over 20% of the variance in TSH and free T4 and that a substantial amount of heritability of thyroid function could be explained by rare variants with larger effects. Results of GWAS for thyroid function are summarized in Table 2.

Thyroid function may be affected by the presence of antibodies to TPO or Tg, even in the normal range. In GWAS of thyroid function, data on the presence of antibodies were limited. Therefore, it is difficult to conclude that the genes found in GWAS of thyroid function determine an individual set point of the hypothalamus-pituitary-thyroid axis. Several genetic loci identified in GWAS of thyroid function were also found in GWAS of autoimmune thyroid diseases (*FOXE1*, *CAPZB*, and *PDE8B*). A detailed examination of the presence of antibodies should be considered when performing GWAS of thyroid function in the future. In addition, only very limited GWAS of thyroid function have been performed in Asians, so more research is needed.

GWAS OF THYROID CANCER

The first GWAS of thyroid cancer was reported in 2009 and showed that common variants located on 9q22.33 (*FOXE1*) and 14q13.3 (NK2 homeobox 1 [*NKX2-1*]) were associated with DTC [41]. Associations at *FOXE1, MBIP*/*NKX2-1*, disrupted in renal carcinoma 3 (*DIRC3*), and *NRG1* have been identified and repeatedly confirmed in individuals of European ancestry [41-44]. Several markers associated with DTC, including inner mitochondrial membrane peptidase subunit 2 (*IMMP2L*), retinoic acid receptor responder 1 (*RARRES1*), small nuclear RNA activating complex polypeptide 4 (*SNAPC4*), basic leucine zipper ATF-like transcription factor (*BATF*), DEAH-box helicase 35 (*DHX35*), UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 4 (*GALNTL4*), 5-hydroxytryptamine receptor 1B (*HTR1B*), forkhead box A2 (*FOXA2*), and WDR11 antisense RNA 1 (*WDR11-AS1*), were identified but not replicated in other studies [43-46]. A recent meta-analysis of GWAS including a total of 3,001 DTC patients and 287,550 controls from five study groups of European populations found five novel loci (pecanex-like 2 [*PCNXL2*], telomerase RNA component [*TERC*], neuronal regeneration related protein [*NREP*]-erythrocyte membrane protein band 4.1 like 4A [*EPB41L4A*], oligosaccharide-binding folds containing 1 [*OBFC1*], and SMAD family member 3 [*SMAD3*]) [47]. Table 3 provides the susceptibility loci identified in GWAS of thyroid cancer [38-40,48,49]. The most robust signals were detected on 9q22.33 (*FOXE1*) in Caucasians [41,50]. The *FOXE1* locus was also reported to be a susceptibility gene for radiation-related thyroid cancer [50]. A functional study showed that common variants on *FOXE1* regulated *FOXE1* transcription through the recruitment of the upstream stimulatory factor 1 (USF1)/USF2 transcription factors [51]. Several reports demonstrated that variants of *FOXE1* were related to aspects of the clinical aggressiveness of papillary thyroid cancer (PTC), such as tumor stage, size, lymphocytic infiltration, and extrathyroidal extension [52,53].

Recently, we reported 15 variants from 11 loci associated with DTC in a Korean GWAS including 1,085 cases of DTC and 8,884 controls [54]. The most robust signals were detected in the *NRG1* gene, and expression quantitative trait loci analysis showed that variants on *NRG1* were also associated with *NRG1* expression in thyroid tissues [54]. He et al. [55] also showed that the expression levels of *NRG1* isoforms were significantly correlated with genotypes. *NRG1* encodes neuregulin-1, which acts on the erb-b2 receptor tyrosine kinase (ERBB) family of tyrosine kinase receptors. In a study of the intrinsic resistance of PTC to a B-Raf inhibitor, ERBB2/ERBB3 activation was found to be dependent on autocrine production of neuregulin-1 [56].

Table 2. Susceptibility Loci for Levels of Thyroid Stimulating Hormone or Free Thyroxine Detected by Genome-Wide Association Studies

Phenotypes	Locus	Gene	Protein function	Population	Reference
TSH	5q13.3	*PDE8B*	Role in hydrolysis of the second messenger cAMP	European, USA, Germany, UK	[38,40,48,49]
	6q27	*PDE10A*	Role in regulation of the intracellular concentration of cyclic nucleotides	European, UK	[38,40]
	1p36.13	*CAPZB*	Regulating actin filament dynamics	European, Chinese Han, Germany, UK	[38-40,49]
	16q23.2	*MAF*	Role in increased T-cell susceptibility to apoptosis	European, UK, Germany	[38,40,49]
	6p21.1	*VEGFA*	Proliferation and migration of vascular endothelial cells	European, UK	[38,40]
	4q31.23	*NR3C2*	Role in aldosterone actions	European, Germany, UK	[38,40,49]
	2q35	*IGFBP5*	Encoding insulin like growth factor binding protein 5	European	[38]
	17q24.3	*SOX9*	Role in chondrocyte differentiation	European	[38]
	1p31.3	*NFIA*	Encoding nuclear factor IA	European	[38]
	15q21.2	*FGF7*	Mitogenic and cell survival activities	European	[38]
	11p11.2	*PRDM11*	Role in transcription regulation	European	[38]
	15q26.1	*MIR1179*	MicroRNA 1179	European	[38]
	19p13.2	*INSR*	Encoding insulin receptor	European	[38]
	9q34.2	*ABO*	Determination of ABO blood group	European, UK	[38,40]
	14q32.12	*ITPK1*	Regulation of the synthesis of inositol tetraphosphate	European	[38]
	8p12	*NRG1*	Role in the growth and development of multiple organ systems	European	[38]
	14q13.3	*MBIP-NKX2-1*	Encoding TTF-1, binding to TG promoter	European, UK	[38,40]
	6q24.3	*SASH1*	Role in the TLR4 signaling pathway	European	[38]
	9p24.2	*GLIS3*	Role in transcription in thyroid gland	European	[38]
	8q12.1	*XKR4*	Role in apoptosis	Chinese Han	[39]
	9q22.33	*FOXE1*	Encoding TTF-2, role in thyroid morphogenesis	Chinese Han, USA, UK	[39,40,48]
	2q35	*IGFBP2*	Encoding insulin like growth factor binding protein 2	UK	[40]
	3p25.2	*SYN2*	Binding to small synaptic vesicles	UK	[40]
Free T4	1p32.3	*DIO1*	Encoding iodothyronine deiodinase 1	European, UK	[38,40]
	9q34.3	*LHX3*	Role in pituitary development	European, UK	[38,40]
	9q22.33	*FOXE1*	Encoding TTF-2, role in thyroid morphogenesis	European	[38]
	4q33	*AADAT*	Role in L-lysine catabolism	European, UK	[38,40]
	16q12.2	*LPCAT2-CAPNS2*	Role in membrane biogenesis	European	[38]
	18q22.3	*NETO1-FBXO15*	Role in spatial learning and memory in the hippocampus	European	[38]
	18q12.1	*B4GALT6*	Role in biosynthesis of glycosphingolipids	UK	[40]

TSH, thyroid stimulating hormone; *PDE8B*, phosphodiesterase 8B; cAMP, cyclic adenosine monophosphate; *PDE10A*, phosphodiesterase 10A; *CAPZB*, capping actin protein of muscle Z-line subunit beta; *VEGFA*, vascular endothelial growth factor A; *NR3C2*, nuclear receptor subfamily 3 group C member 2; *IGFBP5*, insulin like growth factor binding protein 5; *SOX9*, SRY-box 9; *NFIA*, nuclear factor I A; *FGF7*, fibroblast growth factor 7; *PRDM11*, PR/SET domain 11; *MIR1179*, microRNA 1179; *INSR*, insulin receptor; *ITPK1*, inositol-tetrakisphosphate 1-kinase; *NRG1*, neuregulin 1; *MBIP*, MAP3K12 binding inhibitory protein 1; *NKX2-1*, NK2 homeobox 1; TTF, thyroid transcription factor; TG, thyroglobulin; *SASH1*, SAM and SH3 domain containing 1; TLR4, Toll-like receptor 4; *GLIS3*, GLIS family zinc finger 3; *XKR4*, XK related 4; *FOXE1*, forkhead box E1; *IGFBP2*, insulin like growth factor binding protein 2; *SYN2*, synapsin II; T4, thyroxine; *DIO1*, iodothyronine deiodinase 1; *LHX3*, LIM homeobox 3; *AADAT*, aminoadipate aminotransferase; *LPCAT2*, lysophosphatidylcholine acyltransferase 2; *CAPNS2*, calpain small subunit 2; *NETO1*, neuropilin and tolloid like 1; *FBXO15*, F-box protein 15; *B4GALT6*, beta-1,4-galactosyltransferase 6.

NRG1 dysregulation is also closely related with the phosphoinositide 3-kinase (PI3K)-AKT and mitogen-activated protein kinase (MAPK) signaling pathway via ERBB [57]. Our gene set enrichment analysis data showed that variants on *NRG1* were associated with many pathways related to cellular growth or cancer, and the ERBB-MAPK signaling pathway was

Table 3. Susceptibility Loci for Thyroid Cancer Detected by Genome-Wide Association Studies

Locus	Gene	Protein function	Population	References
9q22.33	*FOXE1*	Encoding TTF-2, role in thyroid morphogenesis	Iceland, USA, Spain, Netherlands, Belarus, Italy, Poland, Korea	[41,42, 46-50,54]
14q13.3	*MBIP-NKX2-1*	Encoding TTF-1	Iceland, USA, Spain, Netherlands, Italy, Poland, Korea	[41,42,46, 47,54]
2q35	*DIRC3*	Non-coding RNA	Iceland, USA, Spain, Netherlands, Italy, Poland, UK, Korea	[42,43,47, 54]
8p12	*NRG1*	Role in the growth and development of multiple organ systems	Iceland, USA, Spain, Netherlands, Korea	[42,54]
7q31.1	*IMMP2L*	Catalytic activity of the mitochondrial inner membrane peptidase complex	Italy, Poland, UK, Spain	[43]
3q25.32	*RARRES1*	Encoding a type 1 membrane protein.	Italy, Poland, UK, Spain	[43]
9q34	*SNAPC4*	Role in RNA polymerase II and III transcription from small nuclear RNA promoters.	Italy, Poland, UK, Spain	[43]
14q24.3	*BATF*	Negative regulator of AP-1/ATF transcriptional events	Italy, Poland	[44]
20q11.23	*DHX35*	Putative RNA helicases	Italy, Poland	[44]
5q14	*ARSB*	Role in the regulation of cell adhesion, cell migration and invasion	Italy, Poland, Spain	[44]
13q12	*SPATA13*	Role in regulation of cell migration and adhesion assembly and disassembly	Italy, Poland, Spain	[44]
11p15.3	*GALNTL4*	Role in initial reaction in O-linked oligosaccharide biosynthesis	Italy, Poland, Spain	[45]
20p11	*FOXA2*	Activators for liver-specific genes such as albumin and transthyretin	Italy, Poland, Spain	[45]
10q26.12	*WDR11-AS1*	Non-coding RNA	Italy, Spain	[46]
6q14.1	*HTR1B*	Role in activity of adenylate cyclase and the release of serotonin, dopamine, and acetylcholine	Italy, Spain	[46]
1q42.2	*PCNXL2*	Role in tumorigenesis	Iceland, USA, Spain, Netherlands, Korea	[47,54]
10q24.33	*OBFC1*	Role in initiation of DNA replication	Iceland, USA, Spain, Netherlands	[47]
5q22.1	*NREP-EPB41L4A*	Role in interactions between the cytoskeleton and plasma membrane	Iceland, USA, Spain, Netherlands	[47]
15q22.33	*SMAD3*	Signal transducers and transcriptional modulator	Iceland, USA, Spain, Netherlands	[47]
3q26.2	*TERC-LRRC34*	Encoding telomerase RNA component	Iceland, USA, Spain, Netherlands	[47]
5p15.33	*TERT*	Encoding telomerase reverse transcriptase	Iceland, USA, Spain, Netherlands	[47]
12q14.3	*MSRB3*	Role in reduction of methionine sulfoxide to methionine	Korea	[54]
1p13.3	*VAV3*	Role in actin cytoskeletal rearrangements and transcriptional alterations	Korea	[54]
4q21.1	*SEPT11*	Role in cytokinesis and vesicle trafficking	Korea	[54]
3p14.2	*FHIT*	Role in purine metabolism	Korea	[54]
19p13.2	*INSR*	Encoding insulin receptor	Korea	[54]
12q24.13	*SLC24A6*	Role in cellular calcium homeostasis	Korea	[54]

FOXE1, forkhead box E1; TTF, thyroid transcription factor; *MBIP*, MAP3K12 binding inhibitory protein 1; *NKX2-1*, NK2 homeobox 1; *DIRC3*, disrupted in renal carcinoma 3; *NRG1*, neuregulin 1; *IMMP2L*, inner mitochondrial membrane peptidase subunit 2; *RARRES1*, retinoic acid receptor responder 1; *SNAPC4*, small nuclear RNA activating complex polypeptide 4; *BATF*, basic leucine zipper ATF-like transcription factor; AP-1, activator protein 1; ATF, activating transcription factor; *DHX35*, DEAH-box helicase 35; *ARSB*, arylsulfatase B; *SPATA13*, spermatogenesis associated 13; *GALNTL4*, UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 4; *FOXA2*, forkhead box A2; *WDR11-AS1*, WDR11 antisense RNA 1; *HTR1B*, 5-hydroxytryptamine receptor 1B; *PCNXL2*, pecanex-like 2; *OBFC1*, oligosaccharide-binding folds containing 1; *NREP*, neuronal regeneration related protein; *EPB41L4A*, erythrocyte membrane protein band 4.1 like 4A; *SMAD3*, SMAD family member 3; *TERC*, telomerase RNA component; *LRRC34*, leucine rich repeat containing 34; *TERT*, telomerase reverse transcriptase; *MSRB3*, methionine sulfoxide reductase B3; *VAV3*, vav guanine nucleotide exchange factor 3; *SEPT11*, septin 11; *FHIT*, fragile histidine triad; *INSR*, insulin receptor; *SLC24A6*, solute carrier family 24 member A6.

the most significantly enriched. This evidence indicates that *NRG1* expression in thyroid tissue could contribute to increased DTC risk via ERBB signaling.

Our results confirmed previously reported loci (*FOXE1*, *NKX2-1*, *DIRC3*, and *PCNXL2*) from GWAS of European populations and found novel susceptibility loci (vav guanine nucleotide exchange factor 3 [*VAV3*], INSR, MRSB3, fragile histidine triad [*FHIT*], septin 11 [*SEPT11*], and solute carrier family 24 member A6 [*SLC24A6*]) associated with DTC. Specially, a variant of *SLC24A6* was associated with a specific risk of follicular thyroid cancer, for which the genetic factors that increase the risk of thyroid cancer may vary depending on the cancer subtype. Signals on *VAV3*, *INSR*, *MRSB3*, *FHIT*, *SEPT11*, and *SLC24A6* were only identified in Koreans, suggesting between-study heterogeneity in GWAS of DTC.

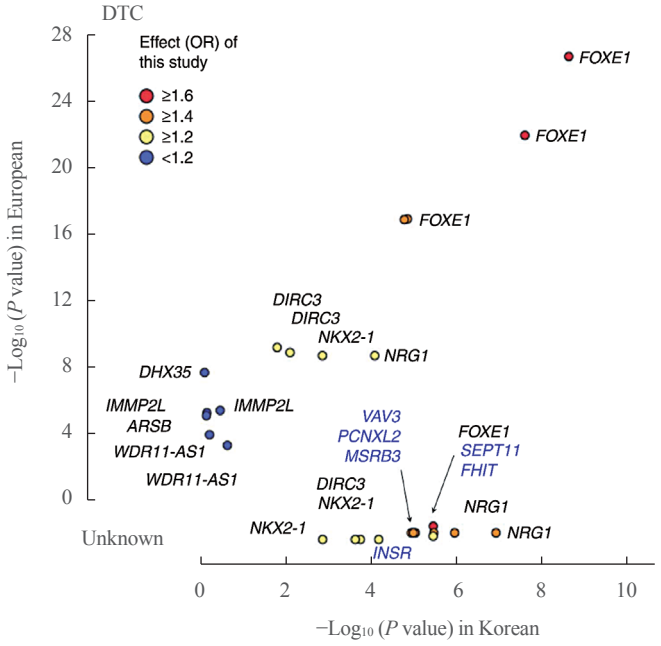

Fig. 1. Comparison of associations between Europeans and Koreans. The *P* values for differentiated thyroid cancer (DTC) between Koreans (x-axis) and Europeans (y-axis) are plotted with the corresponding Korean effect sizes (odd ratio [OR]). The *P* value shows the $-\log_{10}$ scale, and the *P* values of novel single-nucleotide polymorphisms from this study are compared as unknown. Adapted from Son et al. [54]. *FOXE1*, forkhead box E1; *DIRC3*, disrupted in renal carcinoma 3; *NKX2-1*, NK2 homeobox 1; *NRG1*, neuregulin 1; *DHX35*, DEAH-box helicase 35; *IMMP2L*, inner mitochondrial membrane peptidase subunit 2; *ARSB*, arylsulfatase B; *WDR11-AS1*, WDR11 antisense RNA 1; *VAV3*, vav guanine nucleotide exchange factor 3; *PCNXL2*, pecanex-like 2; *MSRB3*, methionine sulfoxide reductase B3; *SEPT11*, septin 11; *FHIT*, fragile histidine triad; *INSR*, insulin receptor.

In GWAS in European and Korean populations, some genetic loci (*FOXE1*, *NKX2-1*, *DIRC3*, *NRG1*, and *PCNXL2*) were commonly found, while certain susceptibility loci were only found in either the European or Korean population. In addition, the risk allele frequency of commonly found SNPs differs by race, and the DTC risk by genotype varies across ethnicities. For example, the risk allele frequencies of variants on *FOXE1* were reported to be 0.14 to 0.34 in Europeans and 0.08 to 0.13 in Asians, suggesting ethnic differences in allele frequencies and a small genetic contribution of variants on *FOXE1* to the development of DTC in East Asians [58]. Moreover, common variants on *FOXE1* were associated with an increased risk of DTC, with an odds ratio (OR) of 1.80 in the European population, but the OR was 1.35 in East Asians [58]. A comparison of these associations, including effect size (OR) and *P* values, between Europeans and Koreans is shown in Fig. 1 [54].

CONCLUSIONS

Twin and family studies of autoimmune thyroid diseases and thyroid cancer have indicated high heritability, suggesting that genetic factors play a key role in disease onset. Previous candidate-gene studies have limitations, such as lack of reproducibility and small sample sizes with limited statistical power. In the last decade, GWAS have unraveled the many forms of genetic predisposition to autoimmune thyroid disease, thyroid function, and thyroid cancer. These genetic discoveries provide insight into the pathogenesis of these diseases and provide opportunities to develop new therapies.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ORCID

Yul Hwangbo *https://orcid.org/0000-0001-7129-2133*
Young Joo Park *https://orcid.org/0000-0002-3671-6364*

REFERENCES

1. Vaidya B, Kendall-Taylor P, Pearce SH. The genetics of autoimmune thyroid disease. J Clin Endocrinol Metab 2002;87:5385-97.
2. Czene K, Lichtenstein P, Hemminki K. Environmental and

heritable causes of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. Int J Cancer 2002;99:260-6.
3. Brix TH, Kyvik KO, Christensen K, Hegedus L. Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. J Clin Endocrinol Metab 2001;86:930-4.
4. Brix TH, Kyvik KO, Hegedus L. A population-based study of chronic autoimmune hypothyroidism in Danish twins. J Clin Endocrinol Metab 2000;85:536-9.
5. Dittmar M, Libich C, Brenzel T, Kahaly GJ. Increased familial clustering of autoimmune thyroid diseases. Horm Metab Res 2011;43:200-4.
6. Tamai H, Ohsako N, Takeno K, Fukino O, Takahashi H, Kuma K, et al. Changes in thyroid function in euthyroid subjects with a family history of Graves' disease: a follow-up study of 69 patients. J Clin Endocrinol Metab 1980;51:1123-7.
7. Hemminki K, Li X, Sundquist J, Sundquist K. The epidemiology of Graves' disease: evidence of a genetic and an environmental contribution. J Autoimmun 2010;34:J307-13.
8. Villanueva R, Greenberg DA, Davies TF, Tomer Y. Sibling recurrence risk in autoimmune thyroid disease. Thyroid 2003;13:761-4.
9. Andersen S, Pedersen KM, Bruun NH, Laurberg P. 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 2002;87:1068-72.
10. Hansen PS, Brix TH, Sorensen TI, Kyvik KO, Hegedus L. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. J Clin Endocrinol Metab 2004;89:1181-7.
11. Panicker V, Wilson SG, Spector TD, Brown SJ, Falchi M, Richards JB, et al. Heritability of serum TSH, free T4 and free T3 concentrations: a study of a large UK twin cohort. Clin Endocrinol (Oxf) 2008;68:652-9.
12. Samollow PB, Perez G, Kammerer CM, Finegold D, Zwartjes PW, Havill LM, et al. Genetic and environmental influences on thyroid hormone variation in Mexican Americans. J Clin Endocrinol Metab 2004;89:3276-84.
13. Park YJ, Ahn HY, Choi HS, Kim KW, Park DJ, Cho BY. The long-term outcomes of the second generation of familial nonmedullary thyroid carcinoma are more aggressive than sporadic cases. Thyroid 2012;22:356-62.
14. Capezzone M, Marchisotta S, Cantara S, Busonero G, Brilli L, Pazaitou-Panayiotou K, et al. Familial non-medullary thyroid carcinoma displays the features of clinical anticipation suggestive of a distinct biological entity. Endocr Relat Cancer 2008;15:1075-81.
15. Ito Y, Kakudo K, Hirokawa M, Fukushima M, Yabuta T, Tomoda C, et al. Biological behavior and prognosis of familial papillary thyroid carcinoma. Surgery 2009;145:100-5.
16. Loh KC. Familial nonmedullary thyroid carcinoma: a meta-review of case series. Thyroid 1997;7:107-13.
17. Uchino S, Noguchi S, Kawamoto H, Yamashita H, Watanabe S, Yamashita H, et al. Familial nonmedullary thyroid carcinoma characterized by multifocality and a high recurrence rate in a large study population. World J Surg 2002;26:897-902.
18. Peiling Yang S, Ngeow J. Familial non-medullary thyroid cancer: unraveling the genetic maze. Endocr Relat Cancer 2016;23:R577-95.
19. Panicker V. Genetics of thyroid function and disease. Clin Biochem Rev 2011;32:165-75.
20. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-78.
21. Simmonds MJ, Howson JM, Heward JM, Cordell HJ, Foxall H, Carr-Smith J, et al. Regression mapping of association between the human leukocyte antigen region and Graves disease. Am J Hum Genet 2005;76:157-63.
22. Simmonds MJ, Howson JM, Heward JM, Carr-Smith J, Franklyn JA, Todd JA, et al. A novel and major association of HLA-C in Graves' disease that eclipses the classical HLA-DRB1 effect. Hum Mol Genet 2007;16:2149-53.
23. Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003;423:506-11.
24. Brand OJ, Barrett JC, Simmonds MJ, Newby PR, McCabe CJ, Bruce CK, et al. Association of the thyroid stimulating hormone receptor gene (TSHR) with Graves' disease. Hum Mol Genet 2009;18:1704-13.
25. Velaga MR, Wilson V, Jennings CE, Owen CJ, Herington S, Donaldson PT, et al. The codon 620 tryptophan allele of the lymphoid tyrosine phosphatase (LYP) gene is a major determinant of Graves' disease. J Clin Endocrinol Metab 2004;89:5862-5.
26. Wellcome Trust Case Control Consortium; Australo-Anglo-American Spondylitis Consortium (TASC), Burton PR, Clayton DG, Cardon LR, Craddock N, et al. Association scan of 14,500 nonsynonymous SNPs in four diseases iden-

tifies autoimmunity variants. Nat Genet 2007;39:1329-37.
27. Chu X, Pan CM, Zhao SX, Liang J, Gao GQ, Zhang XM, et al. A genome-wide association study identifies two new risk loci for Graves' disease. Nat Genet 2011;43:897-901.
28. Cooper JD, Simmonds MJ, Walker NM, Burren O, Brand OJ, Guo H, et al. Seven newly identified loci for autoimmune thyroid disease. Hum Mol Genet 2012;21:5202-8.
29. Denny JC, Crawford DC, Ritchie MD, Bielinski SJ, Basford MA, Bradford Y, et al. Variants near FOXE1 are associated with hypothyroidism and other thyroid conditions: using electronic medical records for genome- and phenome-wide studies. Am J Hum Genet 2011;89:529-42.
30. Eriksson N, Tung JY, Kiefer AK, Hinds DA, Francke U, Mountain JL, et al. Novel associations for hypothyroidism include known autoimmune risk loci. PLoS One 2012;7:e34442.
31. Zhao SX, Xue LQ, Liu W, Gu ZH, Pan CM, Yang SY, et al. Robust evidence for five new Graves' disease risk loci from a staged genome-wide association analysis. Hum Mol Genet 2013;22:3347-62.
32. Chu X, Shen M, Xie F, Miao XJ, Shou WH, Liu L, et al. An X chromosome-wide association analysis identifies variants in GPR174 as a risk factor for Graves' disease. J Med Genet 2013;50:479-85.
33. Kwak SH, Park YJ, Go MJ, Lee KE, Kim SJ, Choi HS, et al. A genome-wide association study on thyroid function and anti-thyroid peroxidase antibodies in Koreans. Hum Mol Genet 2014;23:4433-42.
34. Medici M, Porcu E, Pistis G, Teumer A, Brown SJ, Jensen RA, et al. Identification of novel genetic Loci associated with thyroid peroxidase antibodies and clinical thyroid disease. PLoS Genet 2014;10:e1004123.
35. Matana A, Popovic M, Boutin T, Torlak V, Brdar D, Gunjaca I, et al. Genome-wide meta-analysis identifies novel gender specific loci associated with thyroid antibodies level in Croatians. Genomics 2018 Apr 18 [Epub]. https://doi.org/10.1016/j.ygeno.2018.04.012.
36. Oryoji D, Ueda S, Yamamoto K, Yoshimura Noh J, Okamura K, Noda M, et al. Identification of a Hashimoto thyroiditis susceptibility locus via a genome-wide comparison with Graves' disease. J Clin Endocrinol Metab 2015;100:E319-24.
37. Simmonds MJ. GWAS in autoimmune thyroid disease: redefining our understanding of pathogenesis. Nat Rev Endocrinol 2013;9:277-87.
38. Porcu E, Medici M, Pistis G, Volpato CB, Wilson SG, Cappola AR, et al. A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. PLoS Genet 2013;9:e1003266.
39. Zhan M, Chen G, Pan CM, Gu ZH, Zhao SX, Liu W, et al. Genome-wide association study identifies a novel susceptibility gene for serum TSH levels in Chinese populations. Hum Mol Genet 2014;23:5505-17.
40. Taylor PN, Porcu E, Chew S, Campbell PJ, Traglia M, Brown SJ, et al. Whole-genome sequence-based analysis of thyroid function. Nat Commun 2015;6:5681.
41. Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Sigurdsson A, Bergthorsson JT, et al. Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. Nat Genet 2009;41:460-4.
42. Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Masson G, He H, et al. Discovery of common variants associated with low TSH levels and thyroid cancer risk. Nat Genet 2012;44:319-22.
43. Kohler A, Chen B, Gemignani F, Elisei R, Romei C, Figlioli G, et al. Genome-wide association study on differentiated thyroid cancer. J Clin Endocrinol Metab 2013;98:E1674-81.
44. Figlioli G, Kohler A, Chen B, Elisei R, Romei C, Cipollini M, et al. Novel genome-wide association study-based candidate loci for differentiated thyroid cancer risk. J Clin Endocrinol Metab 2014;99:E2084-92.
45. Figlioli G, Chen B, Elisei R, Romei C, Campo C, Cipollini M, et al. Novel genetic variants in differentiated thyroid cancer and assessment of the cumulative risk. Sci Rep 2015;5:8922.
46. Mancikova V, Cruz R, Inglada-Perez L, Fernandez-Rozadilla C, Landa I, Cameselle-Teijeiro J, et al. Thyroid cancer GWAS identifies 10q26.12 and 6q14.1 as novel susceptibility loci and reveals genetic heterogeneity among populations. Int J Cancer 2015;137:1870-8.
47. Gudmundsson J, Thorleifsson G, Sigurdsson JK, Stefansdottir L, Jonasson JG, Gudjonsson SA, et al. A genome-wide association study yields five novel thyroid cancer risk loci. Nat Commun 2017;8:14517.
48. Malinowski JR, Denny JC, Bielinski SJ, Basford MA, Bradford Y, Peissig PL, et al. Genetic variants associated with serum thyroid stimulating hormone (TSH) levels in European Americans and African Americans from the eMERGE Network. PLoS One 2014;9:e111301.
49. Rawal R, Teumer A, Volzke H, Wallaschofski H, Ittermann T, Asvold BO, et al. Meta-analysis of two genome-wide association studies identifies four genetic loci associated with thyroid function. Hum Mol Genet 2012;21:3275-82.

50. Takahashi M, Saenko VA, Rogounovitch TI, Kawaguchi T, Drozd VM, Takigawa-Imamura H, et al. The FOXE1 locus is a major genetic determinant for radiation-related thyroid carcinoma in Chernobyl. Hum Mol Genet 2010;19:2516-23.
51. Landa I, Ruiz-Llorente S, Montero-Conde C, Inglada-Perez L, Schiavi F, Leskela S, et al. The variant rs1867277 in FOXE1 gene confers thyroid cancer susceptibility through the recruitment of USF1/USF2 transcription factors. PLoS Genet 2009;5:e1000637.
52. Jendrzejewski J, Liyanarachchi S, Nagy R, Senter L, Wakely PE, Thomas A, et al. Papillary thyroid carcinoma: association between germline DNA variant markers and clinical parameters. Thyroid 2016;26:1276-84.
53. Penna-Martinez M, Epp F, Kahles H, Ramos-Lopez E, Hinsch N, Hansmann ML, et al. FOXE1 association with differentiated thyroid cancer and its progression. Thyroid 2014;24:845-51.
54. Son HY, Hwangbo Y, Yoo SK, Im SW, Yang SD, Kwak SJ, et al. Genome-wide association and expression quantitative trait loci studies identify multiple susceptibility loci for thyroid cancer. Nat Commun 2017;8:15966.
55. He H, Li W, Liyanarachchi S, Wang Y, Yu L, Genutis LK, et al. The role of NRG1 in the predisposition to papillary thyroid carcinoma. J Clin Endocrinol Metab 2018;103:1369-79.
56. Montero-Conde C, Ruiz-Llorente S, Dominguez JM, Knauf JA, Viale A, Sherman EJ, et al. Relief of feedback inhibition of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF-mutant thyroid carcinomas. Cancer Discov 2013;3:520-33.
57. Fernandez-Cuesta L, Thomas RK. Molecular pathways: targeting NRG1 fusions in lung cancer. Clin Cancer Res 2015;21:1989-94.
58. Zhu H, Xi Q, Liu L, Wang J, Gu M. Quantitative assessment of common genetic variants on FOXE1 and differentiated thyroid cancer risk. PLoS One 2014;9:e87332.