의학석사 학위논문

한국인에서 전장 유전체 연관 분석을 통한 갑상선 암과 갑상선 결절의 공통 감수성 유전자좌 연구

Genome-wide association study of thyroid nodules in Koreans suggests overlapping susceptibility loci between thyroid cancer and thyroid nodules

2015년 8월

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황보율

한국인에서 전장 유전체 연관 분석을 통한

갑상선 암과 갑상선 결절의 공통 감수성 유전자좌 연구

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황보율

황보율의 의학석사 학위논문을 인준함

2015년 7월

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Abstract (English)

Background

Genome-wide association studies (GWASs) are widely used in human genetics to identify genes associated with various cancers. Several susceptibility loci of differentiated thyroid cancer (DTC) have been identified by GWASs (*FOXE1*, *NKX2-1*, *DIRC3*, *NRG1*, *IMMP2L*, *RARRES1*, and *SNAPC4/CARD9*). However, the relationship of these genetic markers with thyroid nodules has not been evaluated. Additionally, susceptibility loci of thyroid nodules have not been identified.

Objective

Our objective was to identify candidate loci that play a role in thyroid nodules by discovery GWAS.

Methods

We conducted a one-stage case-control GWAS for thyroid nodules in a population-based cohort. Individuals from the Ansung cohort underwent an initial thyroid ultrasonography and a follow-up 2 years later. Additionally, these individuals were evaluated using 1.43 million genotyped or imputed markers. In the two ultrasonographies, 809 individuals showed solid thyroid nodules in both ultrasonographies, while 689 subjects showed normal thyroids in both. We performed logistic regression adjusting for age and sex.

Results

Case-control comparisons identified two independent association signals ($P < 1.0 \times 10^{-5}$): a SNP at 18p11.31 in *EPB41L3* (OR = 1.647, P = 2.09×10^{-7}) and at 10p11.22 in *ITGB1* (OR = 1.645, P = 5.85×10^{-6}). In 13 additional suggestive signals (loci with single-point P values between 1.0×10^{-5} and 5.0×10^{-5}), SNPs were located near *NKX2-1* and *RARRES1*, which are known thyroid cancer susceptibility loci.

Conclusion

We found candidate susceptibility loci for thyroid nodules in a one-stage GWAS. Our findings suggest that thyroid nodules and thyroid cancer share a common genetic etiology.

Keywords: Thyroid nodule, Genome wide association study, Thyroid cancer, Thyroid ultrasonography

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

In Korea, the incidence of thyroid cancer, the most common malignancy, is increasing (1). The majority of thyroid cancers are well-differentiated thyroid cancers (DTCs), which include two subgroups of cancer that are both derived from follicular cells: papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC). PTC is the most common type of thyroid cancer and constitutes over 90% of all thyroid cancers in Korea (2). The recent increase in the incidence of thyroid cancer is mainly due to an increase in the rate of diagnosis of small PTCs, likely because of early diagnosis by high-resolution ultrasonography. Ultrasonography analysis has shown that the prevalence of thyroid nodules is about 40% in the general population (3). The overall risk of malignancy of a thyroid nodule is 5–15% (4).

Thyroid ultrasonography is a useful tool to visualize thyroid nodules. Increase in thyroid nodule size is related to the risk of malignancy (5). Ultrasonographic images aid in the identification of malignant and benign thyroid nodules, specifically malignant thyroid nodules, which are larger and have a taller-than-wide shape, a spiculated margin, marked hypoechogenicity, microcalcification, and macrocalcification (6).

If a thyroid nodule has suspicious features of malignancy by thyroid ultrasonography, fine needle aspiration (FNA) of the thyroid nodule is recommended to confirm the diagnosis (4). In FNA, the needle is inserted into the nodule to collect cells, and the aspirate is stained for cytopathologic examination.

The Bethesda reporting system for FNA cytology is widely used for cytological diagnosis of thyroid lesions (7). However, FNA analysis provides a diagnosis for thyroid nodules in 70–80% cases and about 20–30% of aspirated nodules are diagnosed as indeterminate (4). Several genetic tests have been suggested as additional tools to improve the accuracy of diagnosing malignant thyroid nodules. Recent advances in high-throughput sequencing technologies have enabled the comprehensive characterization of somatic mutations in papillary thyroid cancer (8). These new insights will help in the diagnosis of thyroid cancer by molecular testing for rearrangements, mutation hotspots, and gene expression using FNA specimens.

Despite advances in the diagnosis and treatment for thyroid cancer, there is no established strategy to prevent thyroid cancer. The etiology of thyroid cancer is not well characterized, and genetic and epidemiologic studies for prediction of thyroid cancer are lacking. A better understanding of the pathogenesis of thyroid cancer could aid in the development of preventive strategies. Recently, large-scale genetic studies have been used to study diverse diseases. Specifically, genome-wide association studies (GWASs) have been successful in identifying susceptibility loci for complex diseases by finding common genetic variants associated with the disease. GWASs have the potential to elucidate the pathophysiology of a disease and could lead to the development of new drugs and preventive strategies. GWASs enable the identification of genetic predispositions and gene-environmental interactions.

Recently, several GWASs for thyroid cancer identified single nucleotide

polymorphisms (SNPs) associated with a PTC risk. Case-control GWASs have identified signals near *FOXE1, NKX2-1, DIRC3, NRG1, IMMP2L, RARRES1, SNAPC4/CARD9, BAT,* and *DHX35* as susceptibility loci for DTC or PTC (9-13). DTCs have a high degree of heritability, suggesting that genetic factors can contribute to the pathogenesis of thyroid cancer (14). GWASs have improved our understanding of heritability of thyroid cancer. The cumulative risk of genetic variants could be one of the possible explanations for the high heritability of DTC.

The risk for thyroid nodules is also considered to be heritable and thyroid cancer could be considered a subgroup of thyroid nodules. Thus, documented candidate SNPs for thyroid cancer could actually be a subset of candidate SNPs associated with thyroid nodules. It is unclear whether thyroid cancer and thyroid nodules share a common etiology or whether some thyroid cancers arise from benign nodules. Studies have not yet investigated genetic susceptibility for thyroid nodules or possible common genetic predispositions between thyroid cancer and thyroid nodules. Previous GWASs for thyroid cancer have used the general population for control groups and did not perform thyroid sonography to evaluate thyroid nodules. Thus, genetic susceptibility for thyroid nodules had not been evaluated.

Therefore, we conducted a GWAS to identify potential susceptibility loci for thyroid nodules in a general population-based cohort. We reviewed the results of thyroid cancer GWASs to compare susceptibility loci between thyroid cancer and thyroid nodules.

II. METHODS

1. Study population and thyroid ultrasonography

We performed the discovery stage of a case-control analysis in the Ansung cohort, a community-based cohort living in rural Korea. Details of the Ansung cohort study have been described elsewhere (15). Since 2001, the participants had undergone regular biennial examinations. We performed an initial thyroid ultrasonography between 2011 and 2012 and a follow-up thyroid ultrasonography between 2013 and 2014. Of 5,018 cohort participants, 3,161 individuals were evaluated by thyroid ultrasonography during the first examination. Follow-up ultrasonography was performed in 2,561 of the 3,161 subjects 2 years later. In patients who underwent two consecutive ultrasonographies, 952 individuals had solid nodules and 824 had a normal thyroid gland. The remaining 785 subjects had a pure cystic nodule, disappeared nodule, or newly appeared nodule. Marked hypoechogenicity, taller-than-wide shape, spiculated margins, and calcifications are signs of malignancy. In the 952 individuals with solid nodules, 770 did not have signs of malignancy based on ultrasonography.

2. Genotyping and imputation

DNA was extracted from leukocytes of peripheral blood samples obtained from study individuals. Genotyping was performed using an Affymetrix Genome-Wide

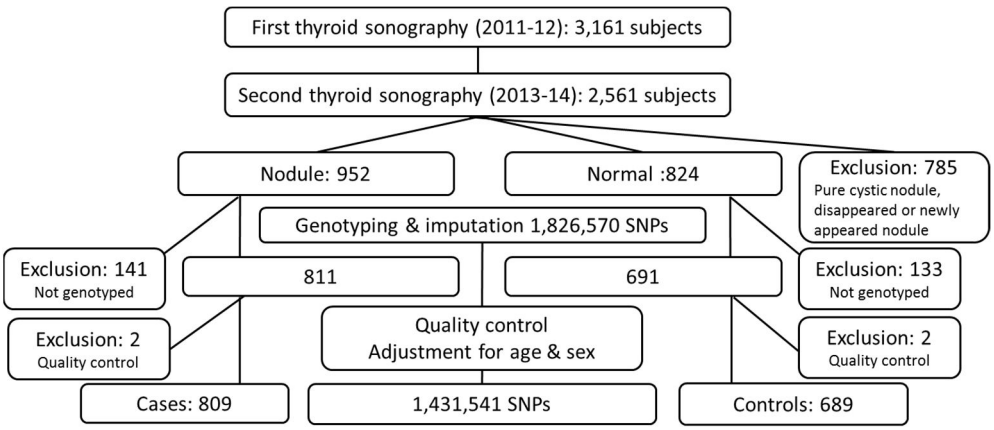
Human SNP Array 5.0. Imputation was carried out using IMPUTE software (https://mathgen.stats.ox.ac.uk/impute). The HapMap phased genotype information of CHB and JPT (build 36 release 22) was used as a reference. Genome positions in NCBI build 36 (UCSC hg 18) were lifted over to NCBI build 37 (UCSC hg19) using binary liftOver tool and python script (http://genome.sph.umich.edu/wiki/LiftOver). We excluded individuals with low call rate (<95%) and SNPs with a minor allele frequency of <5%, SNPs with a missing genotype rate ≥5%, and SNPs whose genotype frequencies departed from Hardy-Weinberg equilibrium at $P < 1 \times 10^{-6}$.

3. Statistical analysis

In the first analysis, 811 of 952 individuals with solid nodules and 691 of 824 individuals with normal thyroids were genotyped. After applying strict quality control criteria, 1,431,541 SNPs were analyzed for association with the risk for solid thyroid nodules in the remaining 809 cases and 689 controls. In the second analysis, genotyping was performed for 656 of 770 individuals who had solid nodules without signs of malignancy and 691 subjects with normal thyroids; 654 individuals with solid nodules were assigned to the case group and 689 individuals with normal thyroids were assigned to the control group after quality control procedures. Statistical analysis was conducted using PLINK version 1.07 and R-software. The genetic analysis package (qqman) for R-CRAN 3.1.3 was used to generate a Manhattan plot and a quantile-quantile (Q-Q) plot. Q-Q plots were used to assess the adequacy of the case-control matching. We also calculated the genomic

inflation factor (λ) from a GWA analysis to compare the genome-wide distribution of the test statistic with the expected null distribution. SNPs for risk of thyroid nodules were analyzed under an additive model. Per-allele odds ratios (ORs) and associated 95% confidence intervals (CIs) were calculated by logistic regression with adjustments for age and sex. We selected the SNPs with high P-values ($P < 5.0 \times 10^{-5}$). Regional plots were generated using the LocusZoom website (http://csg.sph.umich.edu/locuszoom/).

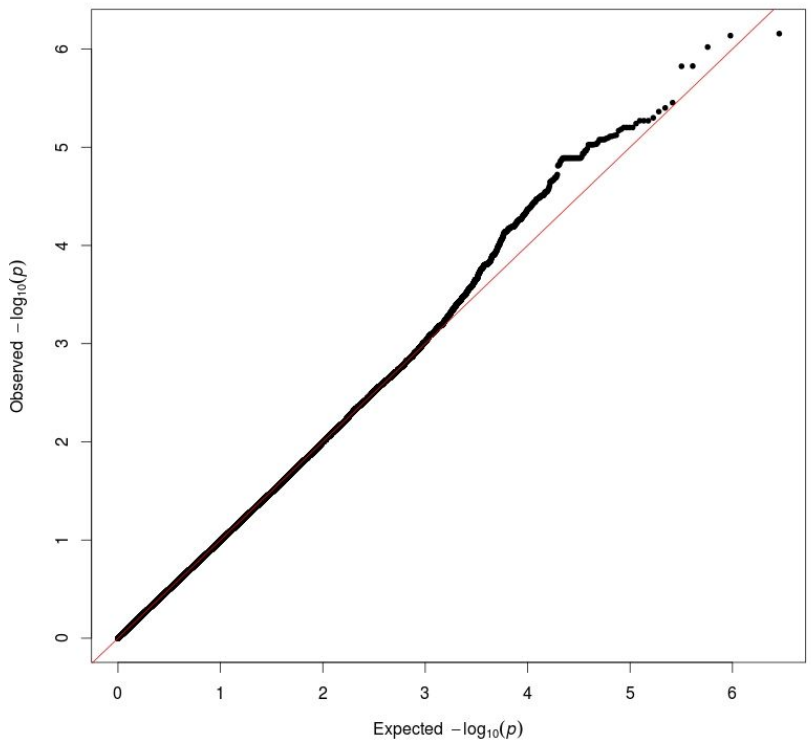
Figure 1. Work flow of this study



III. RESULTS

The Q-Q plot of the observed values of association test statistics compared to the expected distribution showed little deviation from the X = Y line (Figure 1). The inflation factor (λ) was 1.02704 and 1.0 after adjusting for age and sex, suggesting little evidence for inflation of the test statistics.

Figure 2. Comparison of the genome-wide distribution of the test statistic with the expected null distribution: quantile–quantile (Q–Q) plots



We generated a Manhattan plot for the GWAS of thyroid nodules (Figure 3). In the GWAS, eight of the ten strongest signals ($P < 1 \times 10^{-5}$) were located in the same linkage disequilibrium (LD) region of leading SNPs (rs9952940 and rs3780873). SNP rs9952940 is located in an intron of *EPB41L3* (18p11.31) and rs3780873 is in an intron of *ITGB1* (10p11.22). Regional association plots are shown in Figures 4 and 5. Of the 70 SNPs with borderline P-values ($1.0 \times 10^{-5} \leq P < 5.0 \times 10^{-5}$), 13 were lead SNPs. The remaining 57 SNPs had strong LD ($r^2 \geq 0.8$ or $D` \geq 0.8$) with the lead SNPs. Fifteen lead SNPs are shown in Table 1. From 13 suggestive signals, the SNP rs2415317 was located near *NKX2-1*, which is a known thyroid cancer susceptibility locus. The SNPs rs4680504 and rs4680500 are in the *IGCJ-SCHIP1* gene, which is close to the *RARRES1* gene, a susceptibility locus of papillary thyroid cancer identified in a previous GWAS.

Figure 3. Manhattan plot for the GWAS of thyroid nodules

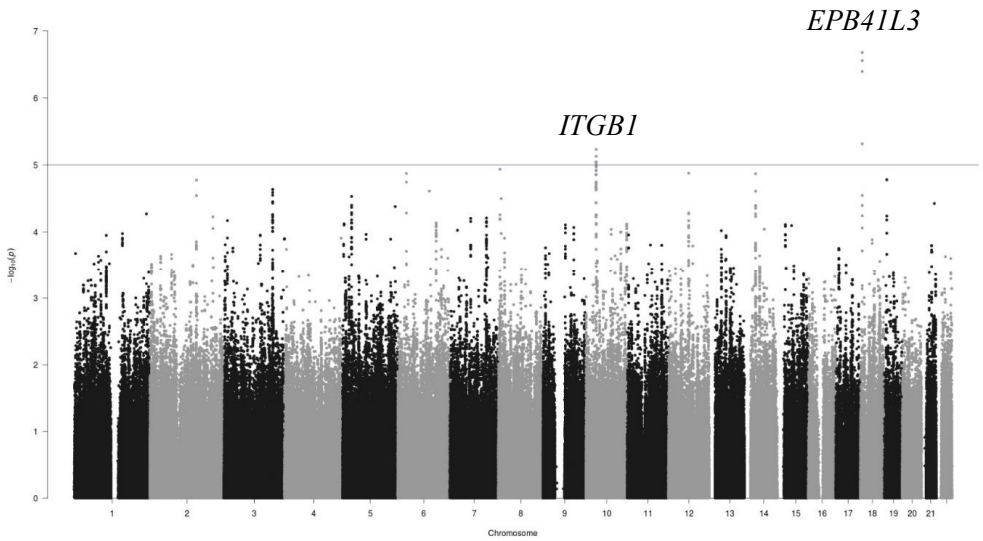


Figure 4. Regional association plot for *EPB41L3 (*18p11.31) across a 400-kb window

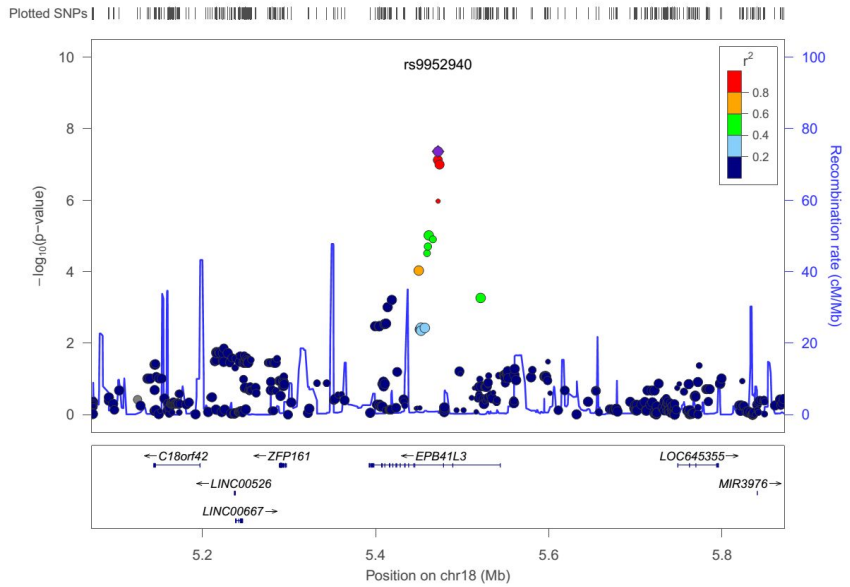


Figure 5. Regional association plot for *ITGB1* (10p11.22) across a 400-kb window

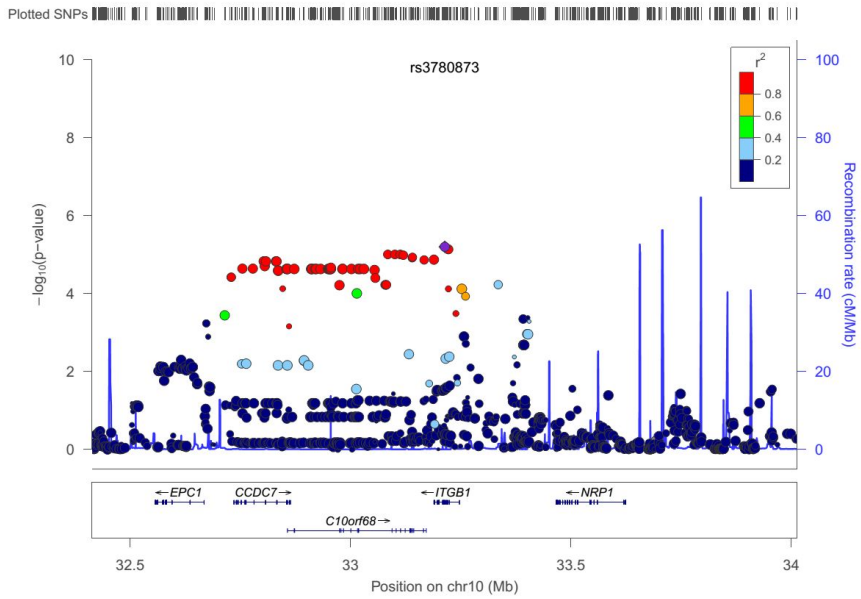


Table 1. Candidate SNPs for thyroid nodules

CHR	SNP	BP	Risk allele	Cases freq	Controls freq	AFR freq	AMR freq	ASN freq	EUR freq	OR (95% CI)	P	Nearby gene	Functional annotation
18	rs9952940	5472021	T	0.809	0.733	0.65	0.75	0.79	0.66	1.647 (1.364-1.988)	2.094×10^{-7}	EPB41L3	Intronic
10	rs3780873	33213680	G	0.880	0.820	0.99	0.92	0.82	0.93	1.645 (1.326-2.040)	5.849×10^{-6}	ITGB1	Intronic
8	rs10282750	5194473	C	0.720	0.652	0.83	0.89	0.68	0.94	1.450 (1.228-1.711)	1.157×10^{-5}	CSMD1	Intergenic
12	rs11175834	65992636	T	0.179	0.125	0.40	0.10	0.14	0.05	1.624 (1.306-2.020)	1.324×10^{-5}	MSRB3	Intronic
6	rs16893827	28197469	T	0.936	0.893	0.86	0.93	0.91	0.94	1.859 (1.406-2.458)	1.335×10^{-5}	ZNF193	Intronic
14	rs2415317	36609678	A	0.488	0.417	0.17	0.44	0.45	0.59	1.406 (1.206-1.640)	1.352×10^{-5}	MBIP/ NKX2-1	Intergenic
10	rs10508774	32807861	A	0.882	0.825	0.91	0.81	0.83	0.91	1.617 (1.302-2.008)	1.388×10^{-5}	CCDC7	Intronic
19	rs17261689	8136027	C	0.178	0.123	0.02	0.14	0.15	0.19	1.610 (1.296-1.999)	1.656×10^{-5}	FBN3	Intronic
2	rs16829835	152123703	A	0.960	0.916	0.84	0.99	0.94	0.99	2.061 (1.483-2.874)	1.677×10^{-5}	NMI	Intergenic
3	rs4680504	159140563	C	0.470	0.408	0.26	0.41	0.46	0.46	1.398 (1.197-1.632)	2.314×10^{-5}	IGCJ-SCHIP1	Intronic
6	rs4543404	103485033	A	0.819	0.760	0.68	0.73	0.83	0.71	1.508 (1.246-1.824)	2.443×10^{-5}	GRIK2	Intergenic
5	rs1864034	29352194	C	0.182	0.133	0.19	0.31	0.14	0.17	1.582 (1.276-1.961)	2.936×10^{-5}	LOC729862	Intergenic
8	rs330010	9127516	G	0.969	0.938	0.50	0.93	0.97	0.99	2.253 (1.537-3.304)	3.163×10^{-5}	LOC157273	Intergenic
21	rs7283308	42101241	A	0.977	0.950	0.93	0.97	0.98	0.95	2.472 (1.608-3.801)	3.741×10^{-5}	DSCAM	Intronic
5	rs17075176	172215412	T	0.653	0.598	0.93	0.75	0.65	0.84	1.403 (1.193-1.649)	4.159×10^{-5}	DUSP1	Intergenic

rs944289 in 14q13.3 is the only SNP associated with thyroid nodules that also has a significant association with a previously identified DTC susceptibility locus (P-value of rs965513 in 9q22.33 (*FOXE1*) = 0.9697; rs966423 in 2q35 (*DIRC3*) = 0.749; rs2439302 in 8p12 (*NRG1*) = 0.171; rs10238549 in 7q31.1 (*IMMP2L*) = 0.8133; rs944289 in 14q13.3 (*NKX2-1*) = 4.051×10^{-5}) (Table 2).

In the second analysis, 654 individuals with solid nodules were assigned to the case group and 689 individuals with normal thyroids were assigned to the control group after quality control procedures. Associated signals with $P < 5.0 \times 10^{-5}$ are described in Table 3. The P-values of the SNPs rs2415317, rs4680504, and rs4680500 were 0.0001385, 0.000175, and 0.0005819, respectively.

Table 2. Documented thyroid cancer-related SNPs

CHR	SNP	BP	Risk allele	Cases freq	Controls freq	AFR freq	AMR freq	ASN freq	EUR freq	OR (95% CI)	P	Nearby gene	Functional annotation
9	rs965513	100556109	A	0.061	0.057	0.10	0.30	0.08	0.34	1.006 (0.725-1.398)	0.970	FOXE1	Intergenic
14	rs944289	36649246	T	0.493	0.427	0.15	0.44	0.45	0.59	1.380 (1.183-1.609)	4.051×10^{-5}	NKX2-1 /MBIP	Intergenic
2	rs966423	218310340	C	0.769	0.762	0.83	0.55	0.76	0.44	1.030 (0.861-1.231)	0.749	DIRC3	Intronic
8	rs2439302	32432369	G	0.220	0.202	0.47	0.49	0.19	0.48	1.139 (0.945-1.373)	0.171	NRG1	Intronic
7	rs10238549	110181022	T	0.148	0.141	0.27	0.25	0.13	0.30	1.026 (0.827-1.273)	0.813	IMMP2L	Intergenic
20	rs7267944	37947434	C	0.356	0.353	0.42	0.20	0.40	0.19	1.032 (0.878-1.213)	0.704	DHX35	Intergenic

Table 3. Candidate SNPs for thyroid nodule without malignant features in ultrasonography

CHR	SNP	BP	Risk allele	Cases freq	Controls freq	AFR freq	AMR freq	ASN freq	EUR freq	OR (95% CI)	P	Nearby gene	Functional annotation
18	rs9952940	5472021	T	0.807	0.733	0.65	0.75	0.79	0.66	1.610 (1.320-1.963)	2.599×10^{-6}	EPB41L3	Intronic
10	rs3780873	33213680	G	0.882	0.820	0.99	0.92	0.82	0.93	1.704 (1.354-2.145)	5.678×10^{-6}	ITGB1	Intronic
8	rs4921697	20116621	C	0.692	0.619	0.92	0.50	0.63	0.58	1.469 (1.238-1.744)	1.060×10^{-6}	LZTS1	Intronic
10	rs10508774	32807861	A	0.884	0.825	0.91	0.81	0.83	0.91	1.673 (1.327-2.108)	1.331×10^{-5}	CCDC7	Intronic
15	rs16958938	47292273	G	0.043	0.015	0.25	0.02	0.03	0.00	3.332 (1.935-5.739)	1.429×10^{-5}	SEMA6D	Intergenic
10	rs11195881	114065993	C	0.562	0.470	0.03	0.34	0.48	0.34	1.424(1.213-1.671)	1.526×10^{-5}	TECTB	Intergenic
5	rs1864034	29352194	C	0.188	0.133	0.19	0.31	0.14	0.17	1.63 (1.303-2.039)	1.856×10^{-5}	LOC729862	Intergenic
6	rs16893827	28197469	T	0.940	0.893	0.86	0.93	0.91	0.94	1.908 (1.411-2.580)	2.676×10^{-5}	ZNF193	Intronic
12	rs10784481	65991148	A	0.568	0.481	0.80	0.44	0.49	0.24	1.415 (1.201-1.667)	3.321×10^{-5}	MSRB3	Intronic

IV. DISCUSSION

Advances in genome-wide association studies are providing new insights into the pathogenesis of differentiated thyroid cancer. Several susceptibility loci of DTC, including 9q22.33 (*FOXE1*), 14q13.3 (*NKX2-1*), 2q35 (*DIRC3*), and 8p12 (*NRG1*), were identified and confirmed by serial GWASs. Additional DTC-associated SNPs were also found nearby, including those in *IMMP2L, RARRES1, SNAPC4/CARD9, BAT,* and *DHX35* (9-13). In those studies, DTC-associated SNPs seemed to be related to the heritability of DTC. Moreover, two studies that assess the cumulative genetic risk of DTC have reported that DTC risk increases with increase in the number of risk alleles (16, 17).

However, most cases of DTC are discovered as a thyroid nodule, which has a reported malignancy risk of 5–10%. Because thyroid nodules are very common in the general population, control groups in previous GWASs included individuals who had thyroid nodules, and DTC could be considered as a subgroup of subjects with thyroid nodules.

Wang et al. reported an association between benign thyroid nodules and a known DTC susceptibility locus, the SNP rs944289T in 14q13.3 (18). This result suggests that some of the susceptibility loci for thyroid cancer might not be cancer specific. Susceptibility loci for DTC in previous GWASs might be associated with thyroid nodules, suggesting that the loci are related with the pathogenesis of thyroid nodules

rather than with thyroid cancer.

In this study, we conducted case-control genome wide analysis for thyroid nodules that were evaluated with ultrasonography. The candidate SNP rs9952940 was located in an intronic region of *EPB41L3* (18p11.31). *EPB41L3* (erythrocyte membrane protein band 4.1-like 3) is a known tumor suppressor that inhibits cell proliferation and promotes apoptosis. This protein is predicted to bind proteins and actin and play a role in the structure of the cytoskeleton. Diseases associated with *EPB41L3* include adenocarcinoma and meningioma. Two studies reported that *EPB41L3* was downregulated in post-Chernobyl and post-radiotherapy-induced thyroid tumors (19, 20). To assess the possible functional role of SNP rs9952940, we utilized the web-based tool HaploReg v2 (www.broadinstitute.org/mammals/haploreg), which is used for the investigation of noncoding genome variants. HaploReg v2 suggests how a SNP affects alterations in regulatory protein binding, histone modifications, chromatin structure, and transcription factor binding sites. Many SNPs in the LD block (SNPs in a strong LD ($r^2 > 0.8$)) were predicted to alter transcription factor binding sites according to HaploReg v2. The risk T allele of rs9952940 is known to alter *FOXA2, FOXD1, FOXJ2, FOXK1,* and *FOXL1*. These transcription factors belong to the forkhead family; there are several reports about the relationship between *FOXA2* and thyroid cancer (21, 22). We examined the expression quantitative trait loci data available for the gene closest to each SNP (cis-eQTL) by using Genevar

(https://www.sanger.ac.uk/resources/software/genevar/). In this search, genetic variants in the LD block containing rs9952940 are not associated with the expression level of *EPB41L3* gene. A functional study of *EPB41L3* in thyroid nodule pathogenesis should be conducted to further investigate its role.

Similarly, SNP rs3780873 was located in an intron of *ITGB1* (10p11.22). In the cis-eQTL analysis, SNPs in the LD block containing rs3780873 are not related to the expression level of *ITGB1*. Cockburn et al. reported that β1 integrin (*ITGB1*) is expressed in the papillary thyroid carcinoma cell line TPC-1 and that RET-induced cell adhesion and migration require *ITGB1* (23). In fact, *RET/PTC* rearrangements were also found in thyroid nodules, especially in post-Chernobyl benign nodules (24). Thus, expression of ITGB1 could play a key role in thyroid nodule formation.

In this study, SNPs located near the *NKX2-1* gene and the *RARRES1* gene were found to have an association with thyroid nodules. After excluding individuals whose sonography findings showed signs of malignant nodules, the association between solid nodules and these SNPs decreased but was still significant. This result suggests that previous reported SNPs in *NKX2-1* and *RARRES1* could be associated with thyroid nodule formation and are not thyroid cancer specific. It is possible that some of the thyroid nodules in this study were thyroid cancer. Thyroid nodules could be associated with loci in *NKX2-1* and *RARRES1,* and a subset of these nodules could be prone to progress to malignancy.

In conclusion, we found 15 candidate SNPs associated with thyroid nodules in the discovery stage of GWAS. The strongest signals were located at 18p11.31 in *EPB41L3* and at 10p11.22 in *ITGB1*. Additional suggestive signals included neighboring SNPs in *NKX2-1* and *RARRES1*. We found that most of the susceptibility loci for thyroid cancer are not related to a risk for thyroid nodules. Our results suggest that there may be some shared genetic markers for thyroid nodules and thyroid cancer. A replication study is needed to confirm these associations in independent populations.

V. REFERENCES

1. **Jung KW, Won YJ, Kong HJ, Oh CM, Cho H, Lee DH, Lee KH** 2015 Cancer Statistics in Korea: Incidence, Mortality, Survival, and Prevalence in 2012. Cancer research and treatment : official journal of Korean Cancer Association

2. **Cho BY, Choi HS, Park YJ, Lim JA, Ahn HY, Lee EK, Kim KW, Yi KH, Chung JK, Youn YK, Cho NH, Park do J, Koh CS** 2013 Changes in the clinicopathological characteristics and outcomes of thyroid cancer in Korea over the past four decades. Thyroid : official journal of the American Thyroid Association 23:797-804

3. **Suk JH, Kim TY, Kim MK, Kim WB, Kim HK, Jeon SH, Shong YK** 2006 Prevalence of ultrasonographically-detected thyroid nodules in adults without previous history of thyroid disease. Journal of Korean Endocrine Society 21:389-393

4. **American Thyroid Association Guidelines Taskforce on Thyroid N, Differentiated Thyroid C, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM** 2009 Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid : official journal of the American Thyroid Association 19:1167-1214

5. **Kamran SC, Marqusee E, Kim MI, Frates MC, Ritner J, Peters H, Benson CB, Doubilet PM, Cibas ES, Barletta J, Cho N, Gawande A, Ruan D, Moore FD, Jr., Pou K, Larsen PR, Alexander EK** 2013 Thyroid nodule size and prediction of cancer. The Journal of clinical endocrinology and metabolism 98:564-570

6. **Moon WJ, Jung SL, Lee JH, Na DG, Baek JH, Lee YH, Kim J, Kim HS, Byun JS, Lee DH, Thyroid Study Group KSoN, Head, Neck R** 2008 Benign and malignant thyroid nodules: US differentiation--multicenter retrospective study. Radiology 247:762-770

7. **Cibas ES, Ali SZ, Conference NCITFSotS** 2009 The Bethesda System For Reporting Thyroid Cytopathology. Am J Clin Pathol 132:658-665

8. **Cancer Genome Atlas Research N** 2014 Integrated genomic characterization of papillary thyroid carcinoma. Cell 159:676-690

9. **Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Sigurdsson A, Bergthorsson JT, He H, Blondal T, Geller F, Jakobsdottir M, Magnusdottir DN, Matthiasdottir S, Stacey SN, Skarphedinsson OB, Helgadottir H, Li W, Nagy R, Aguillo E, Faure E, Prats E, Saez B, Martinez M, Eyjolfsson GI, Bjornsdottir US, Holm H, Kristjansson K, Frigge ML, Kristvinsson H, Gulcher JR, Jonsson T, Rafnar T, Hjartarsson H, Mayordomo JI, de la Chapelle A, Hrafnkelsson J, Thorsteinsdottir U, Kong A, Stefansson K** 2009 Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations.

Nature genetics 41:460-464

10. **Takahashi M, Saenko VA, Rogounovitch TI, Kawaguchi T, Drozd VM, Takigawa-Imamura H, Akulevich NM, Ratanajaraya C, Mitsutake N, Takamura N, Danilova LI, Lushchik ML, Demidchik YE, Heath S, Yamada R, Lathrop M, Matsuda F, Yamashita S** 2010 The FOXE1 locus is a major genetic determinant for radiation-related thyroid carcinoma in Chernobyl. Human molecular genetics 19:2516-2523

11. **Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Masson G, He H, Jonasdottir A, Sigurdsson A, Stacey SN, Johannsdottir H, Helgadottir HT, Li W, Nagy R, Ringel MD, Kloos RT, de Visser MC, Plantinga TS, den Heijer M, Aguillo E, Panadero A, Prats E, Garcia-Castano A, De Juan A, Rivera F, Walters GB, Bjarnason H, Tryggvadottir L, Eyjolfsson GI, Bjornsdottir US, Holm H, Olafsson I, Kristjansson K, Kristvinsson H, Magnusson OT, Thorleifsson G, Gulcher JR, Kong A, Kiemeney LA, Jonsson T, Hjartarson H, Mayordomo JI, Netea-Maier RT, de la Chapelle A, Hrafnkelsson J, Thorsteinsdottir U, Rafnar T, Stefansson K** 2012 Discovery of common variants associated with low TSH levels and thyroid cancer risk. Nature genetics 44:319-322

12. **Kohler A, Chen B, Gemignani F, Elisei R, Romei C, Figlioli G, Cipollini M, Cristaudo A, Bambi F, Hoffmann P, Herms S, Kalemba M, Kula D, Harris S, Broderick P, Houlston R, Pastor S, Marcos R, Velazquez A,**

Jarzab B, Hemminki K, Landi S, Forsti A 2013 Genome-wide association study on differentiated thyroid cancer. The Journal of clinical endocrinology and metabolism 98:E1674-1681

13. **Figlioli G, Kohler A, Chen B, Elisei R, Romei C, Cipollini M, Cristaudo A, Bambi F, Paolicchi E, Hoffmann P, Herms S, Kalemba M, Kula D, Pastor S, Marcos R, Velazquez A, Jarzab B, Landi S, Hemminki K, Forsti A, Gemignani F** 2014 Novel genome-wide association study-based candidate loci for differentiated thyroid cancer risk. The Journal of clinical endocrinology and metabolism 99:E2084-2092

14. **Hemminki K, Eng C, Chen B** 2005 Familial risks for nonmedullary thyroid cancer. The Journal of clinical endocrinology and metabolism 90:5747-5753

15. **Cho NH, Choi HS, Kim KW, Kim HL, Lee SY, Choi SH, Lim S, Park YJ, Park do J, Jang HC, Cho BY** 2010 Interaction between cigarette smoking and iodine intake and their impact on thyroid function. Clinical endocrinology 73:264-270

16. **Liyanarachchi S, Wojcicka A, Li W, Czetwertynska M, Stachlewska E, Nagy R, Hoag K, Wen B, Ploski R, Ringel MD, Kozlowicz-Gudzinska I, Gierlikowski W, Jazdzewski K, He H, de la Chapelle A** 2013 Cumulative risk impact of five genetic variants associated with papillary thyroid carcinoma. Thyroid : official journal of the American Thyroid Association 23:1532-1540

17. **Figlioli G, Chen B, Elisei R, Romei C, Campo C, Cipollini M, Cristaudo A, Bambi F, Paolicchi E, Hoffmann P, Herms S, Kalemba M, Kula D, Pastor S, Marcos R, Velazquez A, Jarzab B, Landi S, Hemminki K, Gemignani F, Forsti A** 2015 Novel genetic variants in differentiated thyroid cancer and assessment of the cumulative risk. Scientific reports 5:8922

18. **Wang YL, Feng SH, Guo SC, Wei WJ, Li DS, Wang Y, Wang X, Wang ZY, Ma YY, Jin L, Ji QH, Wang JC** 2013 Confirmation of papillary thyroid cancer susceptibility loci identified by genome-wide association studies of chromosomes 14q13, 9q22, 2q35 and 8p12 in a Chinese population. Journal of medical genetics 50:689-695

19. **Port M, Boltze C, Wang Y, Roper B, Meineke V, Abend M** 2007 A radiation-induced gene signature distinguishes post-Chernobyl from sporadic papillary thyroid cancers. Radiation research 168:639-649

20. **Ory C, Ugolin N, Levalois C, Lacroix L, Caillou B, Bidart JM, Schlumberger M, Diallo I, de Vathaire F, Hofman P, Santini J, Malfoy B, Chevillard S** 2011 Gene expression signature discriminates sporadic from post-radiotherapy-induced thyroid tumors. Endocrine-related cancer 18:193-206

21. **Kim HS, Kim do H, Kim JY, Jeoung NH, Lee IK, Bong JG, Jung ED** 2010 Microarray analysis of papillary thyroid cancers in Korean. Korean J Intern Med 25:399-407

22. **Akagi T, Luong QT, Gui D, Said J, Selektar J, Yung A, Bunce CM, Braunstein GD, Koeffler HP** 2008 Induction of sodium iodide symporter gene and molecular characterisation of HNF3 beta/FoxA2, TTF-1 and C/EBP beta in thyroid carcinoma cells. Br J Cancer 99:781-788

23. **Cockburn JG, Richardson DS, Gujral TS, Mulligan LM** 2010 RET-mediated cell adhesion and migration require multiple integrin subunits. The Journal of clinical endocrinology and metabolism 95:E342-346

24. **Elisei R, Romei C, Vorontsova T, Cosci B, Veremeychik V, Kuchinskaya E, Basolo F, Demidchik EP, Miccoli P, Pinchera A, Pacini F** 2001 RET/PTC rearrangements in thyroid nodules: studies in irradiated and not irradiated, malignant and benign thyroid lesions in children and adults. The Journal of clinical endocrinology and metabolism 86:3211-3216

초 록

배경: 전장 유전체 연관 분석 (Genome-wide association study, GWAS)은 인간 유전자 연구에서 다양한 암종에서 관련된 유전자를 찾는데 널리 사용되는 방법이다. 분화 갑상선암에서도 전장 유전체 연관 분석을 통하여 몇몇의 유전자가 확인되었다 (*FOXE1*, *NKX2-1*, *DIRC3*, *NRG1*, *IMMP2L*, *RARRES1*, *SNAPC4/CARD9*). 그러나 이러한 유전자와 갑상선 결절과의 관련성은 아직 밝혀지지 않았고 또한 갑상선 결절에 감수성 유전자를 현재까지 확인된 바가 없다.

목적: 본 연구의 목적은 1차 전장 유전체 연관 분석을 통하여 갑상선 결절과 관련된 후보 유전자를 확인하는 것에 있다.

방법: 지역사회 기반 코호트에서 갑상선 결절에 대한 1단계 전장 유전체 연관 분석을 시행하였다. 안성코호트의 연구대상자들에게 갑상선 초음파을 시행하였고 2년후 2차 갑상선 초음파를 시행하였다. 그리고 143만개의 단일염기 다형성 (Single Nucleotide polymorphism, SNP)을 확인하였다. 2번의 초음파에서 809명은 고형결절 소견을 보였고 689명은 정상갑상선 소견을 보여주였다. 이에 성별과 나이를 보정한 로지스틱 회귀분석을 시행하였다

결과: 환자-대조군 비교에서 P값 1.0×10^{-5} 미만의 관련성이 있는 곳은 18p11.31 위치의 *EPB41L3* 유전자와 (OR = 1.647, P = 2.09×10^{-7}) 10p11.22 위치의 *ITGB1* 유전자의 (OR = 1.645, P = 5.85×10^{-6}) SNP이었다. 추가적인 13개의 관련 SNP (P값 1.0×10^{-5} -5.0×10^{-5}사이)중 2개의 SNP이 *NKX2-1* 유전자와 *RARRES1* 유전자에 존재하였고 이 유전자들은 기존 연구에서 갑상선암과 관련된 유전자이다.

결론: 본연구에서 1차 전장 유전체 연관분석을 통하여 갑상선 결절과 관련된 유전자좌를 발견하였다. 이 발견은 갑상선 결절과 갑상선암이 공통의 유전적인 원인을 가질 수 있음을 시사한다.

주요어: 갑상선 결절, 전장 유전체 연관분석, 갑상선암, 갑상선 초음파

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