Genes & Immunity (2019) 20:484–492
https://doi.org/10.1038/s41435-018-0042-z

ARTICLE

Association between thyroglobulin polymorphisms and autoimmune thyroid disease: a systematic review and meta-analysis of case–control studies

Ming-Liang Zhang[1] · Dong-ming Zhang[2] · Cai-E. Wang[1] · Xiao-Long Chen[3] · Fang-Zhou Liu[4] · Jian-Xue Yang[5]

Received: 27 February 2018 / Revised: 11 June 2018 / Accepted: 13 June 2018 / Published online: 24 August 2018

Abstract

Emerging evidence revealed that thyroglobulin (TG) contributes to the development of autoimmune disease, and the relationship between TG and autoimmune thyroid disease (AITD) is still controversial. The aim of this study was to quantify the association between *rs2076740*, *rs853326*, *rs180223*, and *rs2069550* TG polymorphisms and risk of AITD using a meta-analysis approach. We identified all studies that assessed the association between TG polymorphisms and AITD from PubMed, Embase, and Web of Science databases. A total of 3013 cases and 1812 controls from ten case–control studies were included. There was no significant associations found between *rs2069550*, *rs180223*, and *rs853326* polymorphisms and AITD risk. The association between the *rs2076740* polymorphism and AITD risk was significant in the codominant model ($P = 0.005$), suggesting the *CC rs2076740* genotype might be a protective factor for AITD. Sensitivity analysis by removing one or two study changed the results in dominant *rs2076740* and *rs853326* and *rs2069550* allele models ($P =$ 0.016, 0.024, 0.027). Latitude and ethnicity significantly affected the association between *rs2076740* and *rs2069550* polymorphisms and AITD, indicating their protective effects in allele or dominant model ($P = 0.012$, 0.012, 0.012, 0.009, 0.009). The association between *rs2076740, rs2069550*, and *rs853326* polymorphisms and AITD risk is significantly affected by study characteristics.

Introduction

Autoimmune thyroid diseases (AITD) encompass a range of thyroid conditions, including hyperthyroidism (Graves' disease), Hashimoto's disease (Hashimoto's thyroiditis), idiopathic myxedema, and primary hypothyroidism [1–3]. There is solid evidence that interactions between susceptibility genes and environmental triggers activate the sequence of cellular and humoral immune responses to thyroid antigens that cause AITD [4–6]. Several environmental factors, including exposure to excess iodine, selenium deficiency, various infection, certain drugs, and pollutants, have been reported to be associated with AITD [7, 8].

Genetic susceptibility is believed to play a crucial role in this disease etiology [9, 10]. Genetic screening has shown that immune system regulation genes include human leukocyte antigen (HLA) and the cytotoxic T-lymphocyte-associated antigen-4 that are susceptible to AITD [11–13]. Genetic-linkage studies have implicated chromosome 8q24 as a strong susceptibility locus for AITD, sequenced all 48 exons of the thyroglobulin (TG) gene, and identified 14 single-nucleotide polymorphisms (SNPs) [14, 15]. As a 660-kDa glycoprotein, TG provides three things: a thyroid hormone precursor, storage of iodine and of inactive thyroid

✉ Jian-Xue Yang
docyjx1969@126.com

1 Department of Pharmacy, The First Affiliated Hospital and College of Clinical Medicine of Henan University of Science and Technology, Luoyang; The First Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, Henan, China

2 Department of Endocrinology, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, China

3 Department of Infectious Disease, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China

4 Henan Province Chinese Medicine Research Institute, Zhengzhou, Henan, China

5 Department of Neurology, The First Affiliated Hospital and College of Clinical Medicine of Henan University of Science and Technology, Luoyang, China

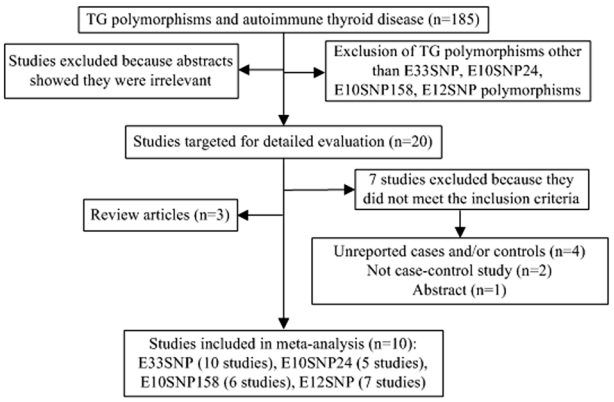

Fig. 1 Flow diagram of the systematic review and meta-analysis literature search results. TG thyroglobulin

hormones, and acts as a major thyroid autoantigen to map to this region in AITD [15, 16].

The most studied polymorphisms in the TG gene are *rs2069550 (E10SNP158)*, *rs853326 (E12SNP)*, and *rs180223 (E10SNP24)* in exons 10–12 and *rs2076740 (E33SNP)* in exon 33 SNP, which were reported to be associated with AITD [17–19]. The same study also found that disease risk increased further when combined with the known HLA susceptibility allele, specifically in the DR3 region [18, 20]. Because TG is an important candidate for one of the major autoantigens for AITD, these data are potentially exciting and are likely to lead to further genetic and functional studies. With evidence for either linkage or association has been investigated in many countries and support from mouse models for thyroiditis [21, 22], the roles for the polymorphisms of TG in AITD risk initially seem confused [5, 18, 23]. Therefore, in order to fully understand the role of TG gene polymorphisms in AITD, it is important to review all of the available data at present. A single study may have low statistical power due to small sample size, single ethnicity, and other limitations, but a comprehensive analysis on different studies with different ethnicities will provide strong evidence on the association of polymorphisms in the TG gene and the risk for AITD. Therefore, we performed this meta-analysis of all eligible studies to investigate the association between TG gene polymorphisms and the risk for AITD. This, to our knowledge, is the first meta-analysis addressing this issue.

Results

Characteristics of studies

Appropriate diagnostic criteria and proper genotyping methods were used in all included studies. Ten case–control studies reporting genotypic frequencies both in cases and in controls were included in meta-analysis [5, 18, 19, 23–29]. Each study character was subdivided into different subgroups. The patient ethnicities included Asians for a majority of studies [5, 19, 24, 26–29]. There were only two populations with European patients [18, 25] and one population with African patients [23]. The study region latitude was subdivided into four subgroups: "21–30°N" [24], "31–40°N" [5, 19, 23, 26–29], "41–50°N" [25], and ">50°N" [18]. Age was subdivided into three subgroups: "≦50 years" [19, 23, 25–29], ">50 years" [5], and unknown [18, 24]. All polymorphisms in the control subjects were in Hardy–Weinberg equilibrium (HWE), except one study for *rs853326* polymorphism [27], one for *rs2076740* polymorphism [19], and one study for *rs2069550* polymorphism and *rs2076740* polymorphis without data [26]. The results of the studies were given in Fig. 1, and study characteristics were summarized in Table 1.

Association between *rs2076740* polymorphism and AITD susceptibility

We firstly analyzed the association between *rs2076740* polymorphism and the susceptibility to AITD in ten case–control studies that included 3013 cases and 1812 controls. Random-effects model was used to detect the study heterogeneity. The analysis showed that *rs2076740* polymorphism was significantly associated with AITD in *CT* vs. *TT* codominant model (odds ratio (OR) = 0.757, $P = 0.005$) (Fig. 2a). The estimated OR1, OR2, and OR3 were 0.606, 0.757, and 0.950, respectively. These estimates suggested a codominant genetic model. The pooled OR1 was 0.606 ($P = 0.058$). There was no evidence of publication bias detected by Egger's test (Egger $P = 0.104$). The sensitivity analysis by removal of two study [23, 26] changed the results in *CC*+*CT* vs. *TT* dominant models. The recalculated OR was 0.683 ($P = 0.016$). These results indicated that the *CC* genotype might be a significant AITD-protective factor compared to the *TT* genotype (Table 2).

The subanalysis showed that AITD risk was significantly reduced in the "31–40°N" group (OR = 0.682, $P = 0.012$) in the *CT* vs. *TT* codominant model, which indicates CT genotype has a protective effect in the "31–40°N" subgroup (Fig. 2b). The results are summarized in Table 3.

Association between *rs180223* polymorphism and AITD susceptibility

The association between *rs180223* polymorphism and the risk of AITD was analyzed in five independent studies with 1896 cases and 1025 controls. Random-effects model was used in the dominant model, codominant model, and allele model due to the presence of heterogeneity, and fixed-effects model was used in the recessive model and homozygous

Table 1 Studies with continuous data on TG polymorphisms in AITD and controls

Reference	TG polymorphisms	Case	Control	HWE (*P*)	Age (case/control)	Genotyping	Country	Racial	Latitude	%Male (case/control)
Ban et al. [29]	*rs2076740*	114	179	0.419	(45.0 ± 10.2)/(43.6 ± 9.8)	PCR-RFLP	Japanese	Asian	35°N	5.4
Collins et al. [18]	*rs180223*	1214	480	0.837	Matched	PCR-RFLP	United Kingdom	European	51°N	100
	rs2069550			0.362						
	rs853326			0.179						
	rs2076740			0.076						
Hsiao et al. [28]	*rs2069550*	215	141	0.020	(40 ±1 3)/(41 ± 12)	PCR-RFLP	China	Asian	33°N	62.46
	rs853326			0.043						
	rs2076740			0.116						
Salima 2008	*rs180223*	108	169	0.440	(13–56)/(18–46)	PCR-RFLP	Tunisian	African	36°N	65.85
	rs853326			0.119						
	rs2076740			0.016						
Maierhaba et al. [27]	*rs180223*	228	131	0.043	(36.66 ± 14.15)/(34.6 ± 13.30)	TaqMan assay	China	Asian	33°N	94.5
	rs2069550			0.088						
	rs853326			0.0002						
	rs2076740			0.292						
Gu et al. [26]	*rs2069550*	436	316	NA	(39.3 ± 13.2)/(49.2 ± 12.7)	Mass-Array™	China	Asian	33°N	100
	rs2076740			NA						
Kotnik et al. [25]	*rs180223*	76	110	0.667	(20.5 ± 5.3)/(20.9 ± 5.3)	TaqMan assay	Slovenia	European	46°N	88.89
	rs2069550			0.667						
	rs853326			0.864						
	rs2076740			0.851						
Wang et al. [19]	*rs180223*	270	135	0.557	(37–38)/(35–38)	PCR-RFLP	China	Asian	33°N	1
	rs2069550			0.809						
	rs853326			0.334						
	rs2076740			0.000						
Patel et al. [24]	*rs2076740*	84	62	0.108	NA	PCR-RFLP	India	Asian	22°N	60.44
Mizuma et al. [5]	*rs853326*	268	89	0.887	>50 (Matched)	PCR-RFLP	Japanese	Asian	35°N	NA
	rs2076740			0.808						

E33SNP, rs2076740; E12SNP, rs853326; E10SNP24, rs180223; E10SNP158, rs2069550

model. The analysis showed that *rs180223* polymorphism was not associated with AITD in any of the studied models. The estimated OR1, OR2, and OR3 were 1.156, 1.442, and 0.884, respectively. These estimates suggest a codominant model, and the pooled OR1 = 1.156 ($P = 0.278$), OR2 = 1.442 ($P = 0.298$), and OR3 = 0.884 ($P = 0.638$). The *TT* genotype was a significant AITD risk factor. There was no heterogeneity in *TT* vs. *GG* ($I^2 = 0\%$). The sensitivity analysis by removal of each individual study did not meaningfully change the results in the codominant model. There was no evidence of publication bias detected by the Egger's test in *TT* vs. *GG* (Egger $P = 0.949$) (Table 2).

Association between *rs2069550* polymorphism and AITD susceptibility

The association between *rs2069550* polymorphism and the risk of AITD was analyzed in six independent studies with 2349 cases and 1433 controls. Fixed-effects model was used in all models due to the presence of heterogeneity. The analysis showed that *rs2069550* polymorphism was not associated with AITD in any of the studied models. The estimated OR1, OR2, and OR3 were 1.108, 0.918, and 1.169, respectively. These estimates suggested a recessive genetic model. The pooled OR was 1.152 ($P = 0.22$). The sensitivity analysis by removal of one study [18] changed the results in *T* vs. *C* allele model. The recalculated OR was 0.844 ($P = 0.024$) (Fig. 3). These results indicated that the *T* genotype might be a significant AITD-protective factor compared to the *C* genotype (Table 2).

The subanalysis showed that people in 31–40°N latitudes significantly reduced AITD risk in both allele model *C* vs. *T* (OR = 0.823, $P = 0.012$) and *TT* + *TC* vs. *CC* dominant model (OR = 0.773, $P = 0.009$) (Fig. 4a, b). The Aisan people with allele model *C* vs. *T* and *TT*+*TC* vs. *CC* dominant model also tended to protect against AITD (OR = 0.799, $P = 0.012$; OR = 0.773, $P = 0.009$) (Fig. 4c, d) (Table 4).

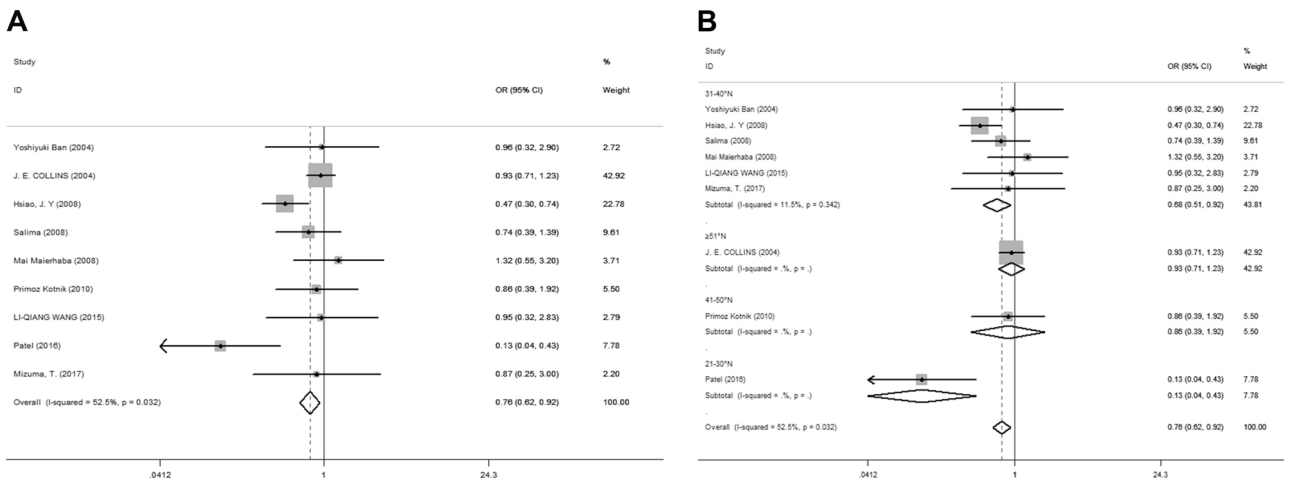

Fig. 2 Meta-analysis with a fixed-effects model for the association between *rs2076740* polymorphism and AITD risk in the codominant model

Association between *rs853326* polymorphism and AITD susceptibility

The association between *rs853326* polymorphism and the risk of AITD was analyzed in seven independent studies with 2379 cases and 1255 controls. Random-effects model was used in the homozygous model, dominant model, codominant model (OR1 and OR2), and allele model due to the presence of heterogeneity, and fixed-effects model was used in the recessive model and codominant model (OR3). The estimated OR1, OR2, and OR3 were 0.854, 0.856, and 1.187, respectively. These estimates suggested a dominant genetic model. The pooled OR was 1.020 ($P = 0.94$). The sensitivity analysis by removal of one study [19] changed the results in $AA + AG$ vs. GG dominant model. The recalculated OR was 1.205 ($P = 0.027$) (Fig. 5a). These results indicated that the *AA* genotype might be a significant AITD risk factor compared to the *GG* genotype (Table 2).

Discussion

The 8q24 region to which the TG gene maps was shown to be strongly linked with AITD [30]. Ban et al. [31] demonstrated that an exon 10–12 SNP cluster and an exon 33 SNP were significantly associated with AITD. However, Collins et al. [18] stated that the SNPs in exons 10, 12, and 33 do not have a causal role for AITD in the United Kingdom in a study of 1214 Caucasian patients in the United Kingdom with AITD. Thus, the association between TG gene polymorphisms and AITD is still controversy. This meta-analysis firstly evaluate the genetic associations between *rs2076740*, *rs180223*, *rs2069550*, and *rs853326* polymorphisms and AITD (GD and HT).

Consistent with Hsiao et al.'s study [28], our analysis clearly revealed the *rs2076740* was significantly associated with AITD in the *CT* vs. *TT* codominant model. This finding suggests that the homozygote CC genotype might be a significant AITD risk factor. In our study, *rs180223*, *rs2069550*, and *rs853326* polymorphisms were not implicated in AITD. However, the sensitivity analysis indicated instability of results and showed significant associations between the dominant model of *rs2076740*, the allele contrast model of *rs2069550*, and the homozygous model of *rs853326* polymorphisms with AITD risk. These results suggest that the TG gene is significantly involved in AITD. Additionally, recent genetic association studies have provided evidence of the involvement of AITD-associated genes and Stefan et al. [32] identified a *−1623A/G* SNP (*rs180195*) in the promoter region of the TG gene that modified a binding site for interferon regulatory factor-1 (IRF-1) or ETS transcriptions factor-1 by combinating the *G* allele in TG at the 8q24.22 region predisposed to AITD. Therefore, AITD is generally considered as the result of interactions among multiple genes.

Considering that AITD is widely accepted as a complex trait that develops in genetically susceptible persons exposed to environmental risk factors [4, 7, 33], we stratified our studies according to study characteristics. Study insufficiency is considered a major limitation when evaluating and comparing all interactions between TG polymorphisms and study characteristics. We found that the associations between *rs2069550* polymorphism and AITD risk was successively dependent on ethnicity. Additionally, we found that 31–40°N latitude significantly affected the association between *rs2076740* and *rs2069550* polymorphisms and AITD risk; it may conform to Martinez's hypothesis [34] that some relationships between genotype and disease will only be observed in conditions of "high"

Table 2 Association between *rs2076740*, *rs853326*, *rs180223*, and *rs2069550* TG polymorphisms and AITD risk

n	Genetic model		OR (95% CI)	*P* value	I^2 (%)	Egger's *P*
rs2076740 (10 studies)	Recessive	*CC* vs. *CT*+*TT*	0.951 (0.716, 1.263)	0.727	61.2	0.472
	Homozygous	*CC*+*TT* vs. *CT*	0.985 (0.854, 1.137)	0.84	32.5	0.882
	Dominant	*CC*+*CT* vs. *TT*	0.870 (0.557, 1.359)	0.541	75.3	0.341
	Codominant (OR1)	*CC* vs. *TT*	0.606 (0.361, 1.017)	0.058	73	0.104
	Codominant (OR2)	*CT* vs. *TT*	0.757 (0.625, 0.917)	**0.005**	**52.5**	0.553
	Codominant (OR3)	*CC* vs. *CT*	0.950 (0.804, 1.122)	0.546	18.7	0.012
	Allele contrast	*C* vs. *T*	0.928 (0.734, 1.173)	0.534	78.3	0.32
rs180223 (4 studies)	Recessive	*TT* vs. *TG*+*GG*	1.154 (0.924, 1.442)	0.206	0	0.599
	Homozygous	*TT*+*GG* vs. *TG*	1.118 (0.900, 1.387)	0.313	0	0.295
	Dominant	*TT*+*TG* vs. *GG*	1.388 (0.727, 2.650)	0.32	89.8	0.373
	Codominant (OR1)	*TT* vs. *GG*	1.156 (0.889, 1.504)	0.278	0	0.949
	Codominant (OR2)	*TG* vs. *GG*	1.442 (0.724, 2.873)	0.298	89.4	0.228
	Codominant (OR3)	*TT* vs. *TG*	0.884 (0.530, 1.475)	0.638	57.8	0.222
	Allele contrast	*T* vs. *G*	1.369 (0.906, 2.071)	0.136	85	0.376
rs2069550 (6 studies)	Recessive	*TT* vs. *TC*+*CC*	1.152 (0.919, 1.443)	0.22	0	0.436
	Homozygous	*TT*+*CC* vs. *TC*	1.123 (0.955, 1.321)	0.159	0	0.818
	Dominant	*TT*+*TC* vs. *CC*	0.872 (0.749, 1.015)	0.077	28.8	0.738
	Codominant (OR1)	*TT* vs. *CC*	1.108 (0.849, 1.447)	0.449	0	0.513
	Codominant (OR2)	*TC* vs. *CC*	0.918 (0.764, 1.102)	0.359	0	0.914
	Codominant (OR3)	*TT* vs. *TC*	1.169 (0.923, 1.481)	0.196	0	0.55
	Allele contrast	*T* vs. *C*	0.948 (0.850, 1.057)	0.334	45.8	0.575
rs853326 (7 studies)	Recessive	*AA* vs. *AG*+*GG*	1.185 (0.983, 1.429)	0.075	0	0.951
	Homozygous	*AA*+*GG* vs. *AG*	1.332 (0.953, 1.862)	0.093	78	0.434
	Dominant	*AA*+*AG* vs. *GG*	1.020 (0.609, 1.708)	0.94	88.9	0.987
	Codominant (OR1)	*AA* vs. *GG*	0.854 (0.515, 1.417)	0.542	67.1	0.448
	Codominant (OR2)	*AG* vs. *GG*	0.856 (0.516, 1.422)	0.549	85.8	0.464
	Codominant (OR3)	*AA* vs. *AG*	1.187 (0.975, 1.445)	0.088	0	0.18
	Allele contrast	*A* vs. *G*	0.872 (0.624, 1.220)	0.425	87.1	0.506

OR odds ratio, *TG* thyroglobulin, *AITD* autoimmune thyroid disease, *n* number of studies, I^2 heterogeneity test

Bold: Significant *P* value (0.05)

rs2076740 polymorphism: Two study removed [23, 29] in the dominant model: OR = 0.683 (95% CI: 0.502–0.931; *P* = 0.016)

rs2069550 polymorphism: One study removed [23] in allele model: recalculated OR = 0.844 (95% CI: 0.729–0.978; *P* = 0.024)

rs853326 polymorphism: One study removed [19] in dominant model: recalculated OR = 1.205 (95% CI: 1.021–1.422; *P* = 0.027)

exposure to an environmental factor of interest and others may only be observed in conditions of "low" exposure. Therefore, genetically predisposed individuals with polymorphisms in genes important for TG metabolism, catabolism, or function has an increased likelihood of developing autoimmune diseases [5, 16, 19].

There are several caveats in this meta-analysis that should be discussed. Most of the group-level data showed large between-study heterogeneity. This could be due to bias, chance, or genuine diversity of genetic effects. Based on Egger's test, we detected a small publication bias. The bias might be caused by unpublished data because negative studies were less likely to be published in journals and were not available in a computerized database, and only studies indexed by the selected databases were included. This results in a potential overestimation of effect sizes. In addition, the smaller published studies did not show different results compared with the larger ones. However, there was a suggestion that the first studies may have yielded somewhat stronger effects. This is consistent with a "winner's curse phenomenon" in which early data show exaggerated effects [35]. Thus, the group-level-derived estimates may be modestly inflated, as suggested also by the trend for relatively smaller effects sizes for the allele of *rs2076740*, *rs180223*, *rs2069550*, and *rs853326* in the individual-level data. The latter may provide more reliable estimators and more options for deciphering the relative contribution of each polymorphism, but they are also not necessarily devoid from potential biases. Nevertheless, genotyping error for SNPs should be low at experienced facilities. Not all cases

Table 3 Association between *rs2076740* polymorphism and AITD: stratification according to study characteristics

Genetic model	Study characteristics	Subgroups	*n*	Association		Heterogeneity	
				OR (95% CI)	*P* value	*I* (%)	*P* value
Codominant (OR2)	Latitude	21–30°N	1	0.133 (0.041, 0.429)	0.001	NA	NA
TT vs. *TC*		31–40°N	6	0.682 (0.506, 0.920)	**0.012**	11.5	0.342
		41–50°N	1	0.864 (0.388, 1.921)	0.719	NA	NA
		≥51°N	1	0.933 (0.706, 1.233)	0.625	NA	NA

Bold significant *P* value ($P < 0.05$)

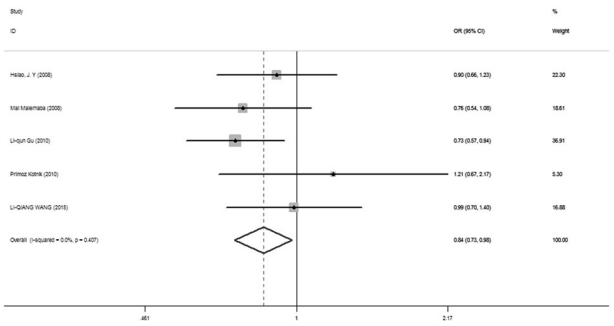

Fig. 3 Meta-analysis with a fixed-effects model for the association between *rs2069550* polymorphism and AITD risk in the allele model

of genotype frequencies were consistent with HWE in the individual-level database, which may be attributed to the mutation of TG. Besides biases, this could be attributed to differences in terms of disease phenotype (e.g. presence of type 1 diabetes and/or of other autoimmune diseases) among AITD cases [25]. AITD is rare in men to allow evaluation of gender differences [36]. Moreover, we detected moderate heterogeneity, which is caused by several factors such as differences in ethnicities. Therefore, the results could be influenced by factors such as random error.

In conclusion, despite these caveats, our collaborative analysis shows consistent associations between AITD with TG. This association crosses ethnic barriers and latitude gradients, and we can make a reasonable estimate of the important role of the TG locus in determining the risk of AITD. However, we still cannot identify a single etiological polymorphism because the current study could not evaluate all interactions between-study characteristics and TG polymorphisms due to insufficient information from the primary publications.

Methods and materials

Identification of eligible studies

The review process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [37]. We performed a literature search in PubMed, Cochrane library, Web of Science and Embase to identify articles that examined the association between *rs2076740*, *rs853326*, *rs180223*, and *rs2069550* TG polymorphisms and AITD (Graves' disease and Hashimoto's thyroiditis) (updated to May 2018). Combinations of keywords, such as "thyroglobulin polymorphisms" and "Autoimmune thyroid disease" were entered as medical subject heading (MeSH) and text words. The reference lists of the articles retrieved were also reviewed to identify publications on the same topic. Two independent reviewers performed searching in duplicate.

Inclusion and exclusion criteria

Studies in this meta-analysis must meet the following inclusion criteria: (1) evaluation of the association between *rs2076740*, *rs853326*, *rs180223*, and *rs2069550* TG polymorphisms and the AITD risk; (2) case–control study; (3) studies focusing on human being; (4) detailed genotype data could be acquired to calculate the ORs and 95% confidence intervals (CIs); Exclusion criteria: (1) duplication of previous publications; (2) comment, review and editorial; (3) family-based studies of pedigrees; (4) study with no detailed genotype data. Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract and full-text. Any dispute was solved by discussion.

Data extraction

The data of the eligible studies were extracted in duplicate by two investigators independently (Zhang and Chen). The following contents were collected: name of first author, year of publication, the characteristics of cases and controls, country of origin, the detective sample, ethnicity, genotyping methods, HWE, number of cases and controls in case and control for *rs2076740*, *rs853326*, *rs180223* and *rs2069550* genotypes, respectively. Different ethnicity descents were classified as Caucasian and Asian. Two authors checked the extracted data and reached to consensus on all the data. If a dissent existed, they would recheck the original data of the included studies and have a discussion to reach consensus. If the dissent still existed, the third investigators would be involved to adjudicate the disagreements (Wang).

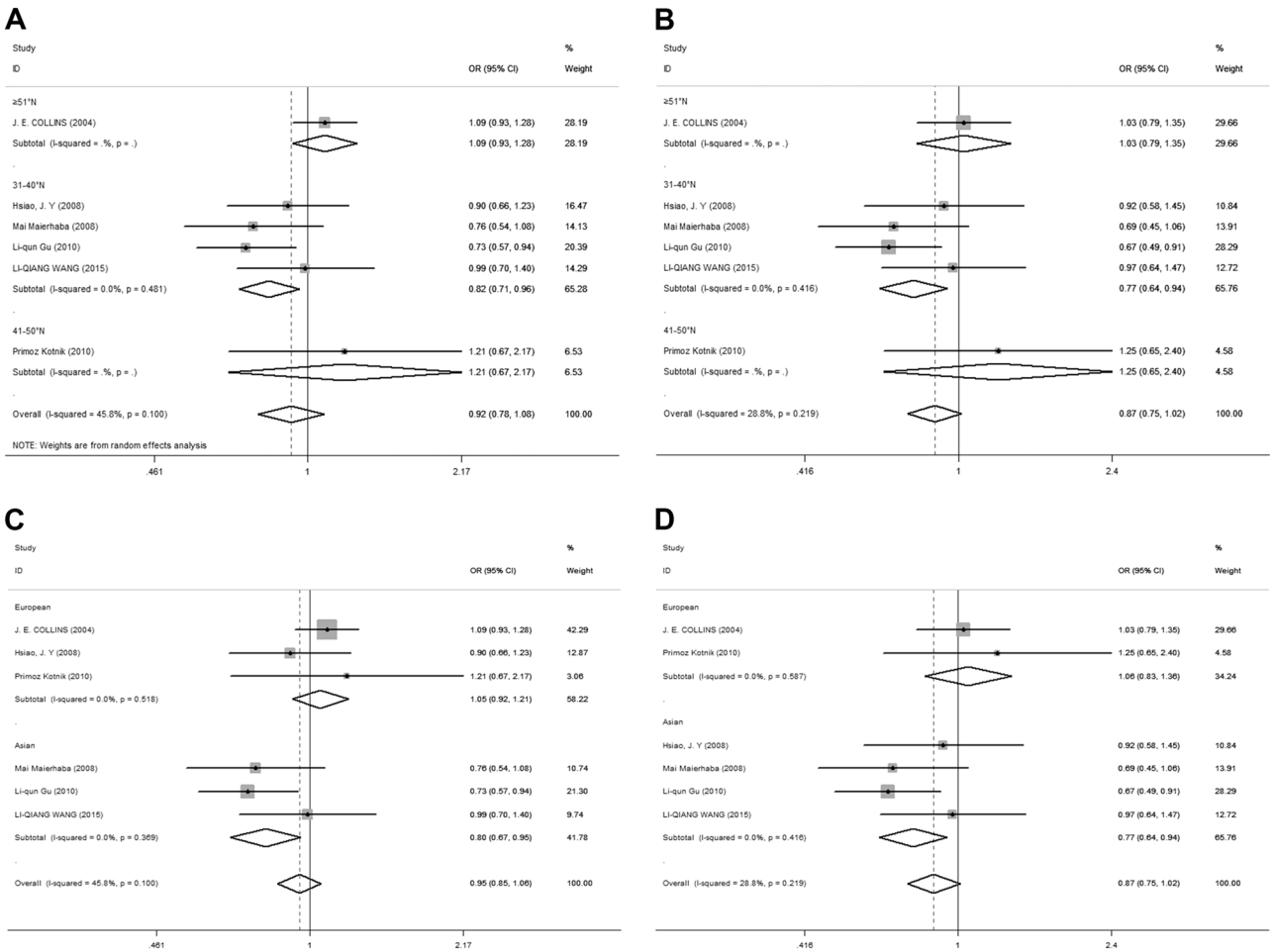

Fig. 4 Meta-analysis with a fixed-effects model for the association between *rs2069550* polymorphism and AITD risk in the allele and dominant model

Statistics analysis

The data from studies were combined to produce a summary OR and represented as an estimated value and 95% confidence intervals (CIs) on a forest plot. The methodology for meta-analysis of molecular studies was described by Thakkinstian et al. [38]. The OR1, OR2, and OR3 values were calculated for the following genotypes: (i) *CC* vs. *TT* (OR1), *CT* vs. *TT* (OR2), and CC vs. *CT* (OR3) for the *rs2076740* polymorphism; (ii) *TT* vs. *GG* (OR1), *TG* vs. *GG* (OR2) and *TT* vs. *TG* (OR3) for *rs180223* polymorphism; (iii) *TT* vs. *CC* (OR1), *TC* vs. *CC* (OR2), and *TT* vs. *TC* (OR3) for *rs2069550* polymorphism; and (iv) *AA* vs. *GG* (OR1), *AG* vs. *GG* (OR2), and *AA* vs. *AG* (OR3) for the *rs853326* polymorphism. The pairwise differences were used to indicate the most appropriate genetic model as follows: if OR1 = OR3≠1 and OR2 = 1, then a recessive model was suggested; if OR1 = OR2≠1 and OR3 = 1, then a dominant model was suggested; if OR2 = 1/OR3≠1 and OR1 = 1, then a complete overdominant model (homozygous) was suggested; if OR1 > OR2 > 1 and OR1 > OR3 > 1 (or OR1 < OR2 < 1 and OR1 < OR3 < 1), then a codominant model was suggested [38]. The data heterogeneity was evaluated using the *Q*-statistic [39]. When the significant *Q*-statistic indicated heterogeneity across studies, then a random-effects model was used. I^2 values of 25%, 50% and 75% were defined as low, moderate, and high estimates, respectively. A symmetric plot and the *P* value of Egger's test <0.05 was considered a significant publication bias [40]. The subgroup analysis was planned when sufficient information was reported in at least two studies in each subgroup. The stability of the summary risk estimate was evaluated using a sensitivity analysis in which each study was individually removed and the OR was recalculated. All statistical analyses were performed with the Stata 12.0 software (StataCorp, College Station, TX, USA). A two-tailed $P < 0.05$ was considered as significant except for specified conditions, where a certain *P* value was declared.

Table 4 Association between *rs2069550* polymorphism and AITD: stratification according to study characteristics

Genetic model	Study characteristics	Subgroups	*n*	Association		Heterogeneity	
				OR (95% CI)	*P* value	*I* (%)	*P* value
Allele contrast	Ethnicity	European	3	1.054 (0.917, 1.212)	0.456	0	0.518
C vs. *T*		Asian	3	0.799 (0.670, 0.952)	**0.012**	0	0.369
	Latitude	31–40°N	4	0.823 (0.706, 0.959)	**0.012**	0	0.481
		41–50°N	1	1.206 (0.670, 2.171)	0.532	NA	NA
		≥51°N	1	1.089 (0.926, 1.281)	0.302	NA	NA
	%Male (case/control)	≦50	1	0.988 (0.699, 1.397)	0.945	56.4	0.057
		>50	5	0.943 (0.841, 1.058)	0.32	NA	NA
Dominant	Ethnicity	European	2	1.060 (0.829, 1.356)	0.642	0	0.587
TT+*TC* vs. *CC*		Asian	4	0.773 (0.638, 0.938)	**0.009**	0	0.416
	Latitude	31–40°N	4	0.773 (0.638, 0.938)	**0.009**	0	0.416
		41–50°N	1	1.253 (0.653, 2.403)	0.498	NA	NA
		≥51°N	1	1.030 (0.789, 1.346)	0.826	NA	NA
	%Male (case/control)	≦50	1	0.971 (0.624, 1.469)	0.89	NA	NA
		>50	5	0.857 (0.728, 1.010)	0.065	40.4	0.152

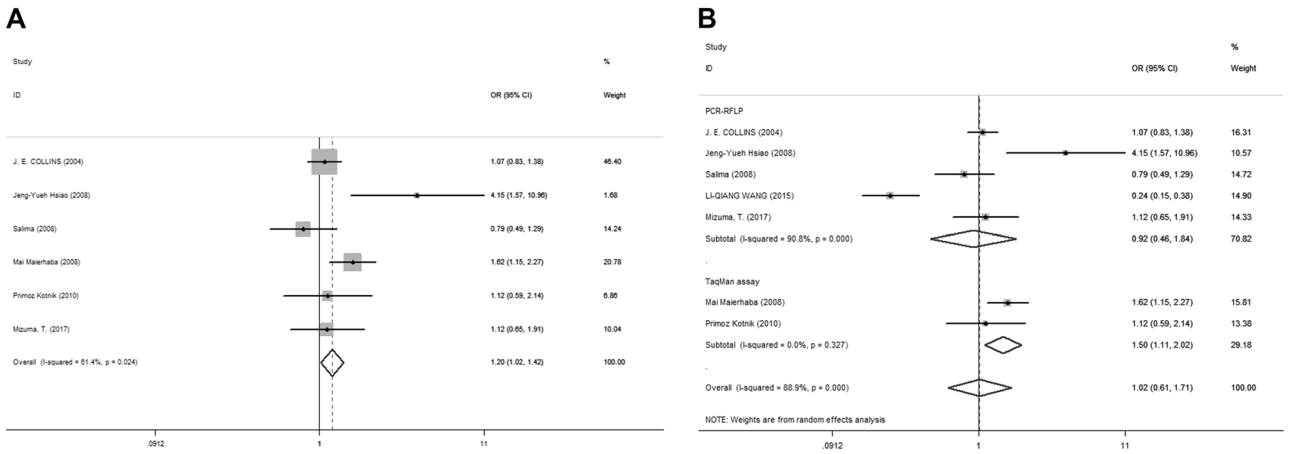

Fig. 5 Meta-analysis with a fixed-effects model for the association between *rs853326* polymorphism and AITD risk in the dominant model

Acknowledgements This study was supported by the National Natural Science Foundation of China (no. 31570357).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Wang B, Shao X, Song R, Xu D, Zhang JA. The emerging role of epigenetics in autoimmune thyroid diseases. Front Immunol. 2017;8:396.
2. Coppede F. Epigenetics and autoimmune thyroid diseases. Front Endocrinol (Lausanne). 2017;8:149.
3. Brown RS. Autoimmune thyroid disease: unlocking a complex puzzle. Curr Opin Pediatr. 2009;21:523–8.
4. Ferrari SM, Fallahi P, Antonelli A, Benvenga S. Environmental issues in thyroid diseases. Front Endocrinol (Lausanne). 2017;8:50.
5. Mizuma T, Watanabe M, Inoue N, Arakawa Y, Tomari Tomari, Hidaka Y. et al. Association of the polymorphisms in the gene encoding thyroglobulin with the development and prognosis of autoimmune thyroid disease. Autoimmunity. 2017;50:386–92.
6. Balazs C. The role of hereditary and environmental factors in autoimmune thyroid diseases. Orv Hetil. 2012;153:1013–22.
7. Wiersinga WM. Clinical relevance of environmental factors in the pathogenesis of autoimmune thyroid disease. Endocrinol Metab (Seoul). 2016;31:213–22.
8. Guarneri F, Benvenga S. Environmental factors and genetic background that interact to cause autoimmune thyroid disease. Curr Opin Endocrinol Diabetes Obes. 2007;14:398–409.
9. Kus A, Arlukowicz-Grabowska M, Szymanski K, Wunsch E, Milkiewicz M, Ploski R, et al. Genetic risk factors for autoimmune thyroid disease might affect the susceptibility to and modulate the progression of primary biliary cholangitis. J Gastrointestin Liver Dis. 2017;26:245–52.

10. Tomer Y. Genetic susceptibility to autoimmune thyroid disease: past, present, and future. Thyroid. 2010;20:715–25.
11. Ramgopal S, Rathika C, Padma MR, Murali V, Arun K, Kamaludeen MN, et al. Interaction of HLA-DRB1* alleles and CTLA4 (+49 AG) gene polymorphism in autoimmune thyroid disease. Gene. 2017;642:430–8.
12. Xiaoheng C, Yizhou M, Bei H, Huilong L, Xin W, Rui H, et al. General and specific genetic polymorphism of cytokines-related gene in AITD. Mediat Inflamm. 2017;2017:3916395.
13. Ting WH, Chien MN, Lo FS, Wang CH, Huang CY, Lin CL, et al. Association of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) gene polymorphisms with autoimmune thyroid disease in children and adults: case–control study. PLoS ONE. 2016;11: e0154394.
14. Ban Y, Tozaki T, Taniyama M, Skrabanek L, Nakano Y, Ban Y, et al. Multiple SNPs in intron 41 of thyroglobulin gene are associated with autoimmune thyroid disease in the Japanese population. PLoS ONE. 2012;7:e37501.
15. Rivolta CM, Targovnik HM. Molecular advances in thyroglobulin disorders. Clin Chim Acta. 2006;374:8–24.
16. Kong YM, Brown NK, Morris GP, Flynn JC. The essential role of circulating thyroglobulin in maintaining dominance of natural regulatory T cell function to prevent autoimmune thyroiditis. Horm Metab Res. 2015;47:711–20.
17. Gough S. The thyroglobulin gene: the third locus for autoimmune thyroid disease or a false dawn? Trends Mol Med. 2004;10:302–5.
18. Collins JE, Heward JM, Howson JM, Foxall H, Carr-Smith J, Franklyn JA, et al. Common allelic variants of exons 10, 12, and 33 of the thyroglobulin gene are not associated with autoimmune thyroid disease in the United Kingdom. J Clin Endocrinol Metab. 2004;89:6336–9.
19. Wang LQ, Wang TY, Sun QL, Qie YQ. Correlation between thyroglobulin gene polymorphisms and autoimmune thyroid disease. Mol Med Rep. 2015;12:4469–75.
20. 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.
21. Jacobson EM, Concepcion E, Ho K, Kopp P, Vono Toniolo J, Tomer Y. cDNA immunization of mice with human thyroglobulin generates both humoral and T cell responses: a novel model of thyroid autoimmunity. PLoS ONE. 2011;6:e19200.
22. Kolypetri P, Noel NA, Carayanniotis KA, Carayanniotis G. Iodine content of thyroglobulin in Nod.H2h4 mice developing iodine-accelerated autoimmune thyroiditis. Hormones (Athens). 2010;9:151–60.
23. Belguith-Maalej S, Hadj Kacem H, Rebai A, Mnif M, Abid M, Ayadi H. Thyroglobulin polymorphisms in Tunisian patients with autoimmune thyroid diseases (AITD). Immunobiology. 2008;213:577–83.
24. Patel H, Mansuri MS, Singh M, Begum R, Shastri M, Misra A. Association of cytotoxic T-lymphocyte antigen 4 (CTLA4) and thyroglobulin (TG) genetic variants with autoimmune hypothyroidism. PLoS ONE. 2016;11:e0149441.
25. Kotnik P, Debeljak M, Avbelj M, Hovnik T, Ursic Bratina N, Krzisnik C, et al. Lack of association of common allelic variants in the thyroglobulin gene with Hashimoto's thyroiditis in young subjects with type 1 diabetes. Horm Res Paediatr. 2010; 73:244–7.
26. Gu LQ, Zhu W, Zhao SX, Zhao L, Zhang MJ, Cui B, et al. Clinical associations of the genetic variants of CTLA4, Tg, TSHR, PTPN22, PTPN12 and FCRL3 in patients with Graves' disease. Clin Endocrinol (Oxf). 2010;72:248–55.
27. Maierhaba M, Zhang JA, Yu ZY, Wang Y, Xiao WX, Quan Y, et al. Association of the thyroglobulin gene polymorphism with autoimmune thyroid disease in Chinese population. Endocrine. 2008;33:294–9.
28. Hsiao JY, Tien KJ, Hsiao CT, Hsieh MC. Exon 33 T/T genotype of the thyroglobulin gene is a susceptibility gene for Graves' disease in Taiwanese and exon 12 C/C genotype protects against it. Clin Exp Med. 2008;8:17–21.
29. Ban Y, et al. Association of a thyroglobulin gene polymorphism with Hashimoto's thyroiditis in the Japanese population. Clin Endocrinol (Oxf). 2004;61:263–8.
30. Tomer Y, Greenberg D. The thyroglobulin gene as the first thyroid-specific susceptibility gene for autoimmune thyroid disease. Trends Mol Med. 2004;10:306–8.
31. Ban Y, Tozaki T, Taniyama M, Tomita M, Ban Y. Amino acid substitutions in the thyroglobulin gene are associated with susceptibility to human and murine autoimmune thyroid disease. Proc Natl Acad Sci USA. 2003;100:15119–24.
32. Stefan M, Jacobson EM, Huber AK, Greenberg DA, Li CW, Skrabanek L, et al. Novel variant of thyroglobulin promoter triggers thyroid autoimmunity through an epigenetic interferon alpha-modulated mechanism. J Biol Chem. 2011;286: 31168–79.
33. Brent GA. Environmental exposures and autoimmune thyroid disease. Thyroid. 2010;20:755–61.
34. Martinez FD. Gene-environment interaction in complex diseases: asthma as an illustrative case. Novartis Found Symp. 2008;293:184–92. discussion192–7
35. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. Nat Genet. 2001;29:306–9.
36. Manji N, Carr-Smith JD, Boelaert K, Allahabadia A, Armitage M, Chatterjee VK, et al. Influences of age, gender, smoking, and family history on autoimmune thyroid disease phenotype. J Clin Endocrinol Metab. 2006;91:4873–80.
37. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6:e1000097.
38. Thakkinstian A, McElduff P, D'Este C, Duffy D, Attia J. A method for meta-analysis of molecular association studies. Stat Med. 2005;24:1291–306.
39. Tizaoui K, Kaabachi W, Hamzaoui A, Hamzaoui K. Association between vitamin D receptor polymorphisms and multiple sclerosis: systematic review and meta-analysis of case–control studies. Cell Mol Immunol. 2015;12:243–52.
40. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–34.