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ARTICLE

IRAK2 and *TLR10* confer risk of Hashimoto's disease: a genetic association study based on the Han Chinese population

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Abstract

Hashimoto's disease (HD) is one of the major clinical subtypes of autoimmune thyroid disease. Both environmental and genetic factors contribute to the pathogenesis of HD. Previous evidence has shown that both *IRAK2* and *TLR10* are potential candidate susceptibility genes for HD. In this study, a total of 3654 Chinese women, including 973 HD cases and 2681 healthy controls, were recruited. Thirty-three tag single nucleotide polymorphisms (SNPs) in *IRAK2* and *TLR10* were genotyped. Genetic association analyses at both the single-marker and haplotype levels were performed. Gene-by-gene interaction analyses were also conducted in case-only samples, as well as eQTL analyses for significant SNPs based on data extracted from the GTEx database. We identified that two SNPs, rs165501 (OR = 1.20, $P = 0.0008$, *IRAK2*) and rs10004195 (OR = 1.23, $P = 0.0001$, *TLR10*), were identified to be significantly associated with HD. Rs10004195 was significantly associated with the gene expression of *TLR10* in human pituitary tissues ($P = 2.00 \times 10^{-4}$), while rs165501 was significantly associated with the expression of *IRAK2* in human thyroid tissues ($P = 3.10 \times 10^{-6}$). No significant results were obtained in the gene-by-gene interaction analyses. Our findings suggest that both *IRAK2* and *TLR10* play important roles in the onset and development of HD.

Introduction

Autoimmune thyroid disease (AITD) constitutes 30% of all autoaggressive disorders, including two main clinical subtypes: Graves' disease (GD) and Hashimoto's disease (HD) [1]. HD is more prevalent in women, affecting approximately 5% of the population at some point in their lives [2]. No symptoms are present in the early stage of HD, but painless goiter, hypothyroidism (weight gain, fatigue, constipation, depression, and general pain) and thyroid atrophy develop with the progress of the disease. Morphologically, HD consists of a gradual atrophy of the thyroid tissue following gland invasion with lymphocytic cells, follicular atrophy, and hyperemia accompanied by oncocytic metaplasia of the follicular cells [3]. Thyroid lymphoma is one of the potential complications of HD, but the pathogenesis linking these diseases remains unclear [4]. While both environmental and genetic factors contribute to the pathogenesis of HD, studies of monozygotic twins and CTLA-4 gene polymorphisms have presented strong evidence for a largely genetic etiology of HD [5, 6]. Although these studies confirmed genetic components of HD [7], the etiology and pathogenesis, including finding susceptibility genes for HD, still urgently requires investigation.

With the development of genetic analysis and high-throughput sequencing, more and more susceptibility variants of complex diseases have been identified [8–14].

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Scientists have provided supportive evidence for the polygenic nature of HD and identified some genes, such as *SH2B3*, associated with HD etiology [15]. Recently, a study investigating polymorphisms in 85 Korean AITD patients and 279 healthy control subjects suggested that Toll-like receptor 10 (*TLR10*) polymorphisms may contribute to the pathogenesis of AITD [16]. Moreover, *TLR10* mRNA was significantly increased in the peripheral blood mononuclear cells of AITD patients compared with those of controls [17], further strengthening the association between *TLR10* and AITD. However, these results can explain only a small portion of HD pathogenesis, and the relationship between TLR10 and HD remains unknown. TLRs are a group of transmembrane proteins in humans [18] that act as the primary receptors of innate immunity and are major factors in the pathogenesis of inflammatory diseases, injuries, and cancers. TLR10 is encoded by the *TLR10* gene, which is located on chromosome 4p14; it is distinct from other TLRs, having an anti-inflammatory function, and is associated with prostate cancer, asthma and IgA nephropathy [19–21]. However, reports of possible associations between *TLR10* polymorphisms and HD have been rare to date. TLRs trigger an innate immune response by activating signaling pathways dependent on interleukin-1 receptor-associated kinase (IRAK), and IRAK2 is critical in late-phase TLR responses and the initial responses to TLR stimulation [22, 23]. The IRAK2 protein, which is encoded by the *IRAK2* gene located at 3p25.3, is one of two putative serine/threonine kinases that become associated with the interleukin-1 receptor (IL1R) upon stimulation. A previous study has shown that sequence variants in the IRAK1-MECP2 region confer susceptibility to the AITDs GD and HD [24]. However, to the best of our knowledge, no report exists of suspicious associations between *IRAK2* and HD or AITD. The information presented here suggests that polymorphisms in *TLR10* and *IRAK2* may contribute to the pathogenesis of HD, and this possibility requires further study.

With evidence of significant associations between *TLR10* and AITD in Korean children [16], HD, as one type of AITD, might also be associated with the *TLR10* gene. Therefore, the contributions of TLR10 and IRAK2, a key regulator of the TLR family, to HD risk deserve exploration and study. To further investigate the associations of *TLR10* and *IRAK2* with the risk of HD, we performed a hospital-based case-control study to further identify the associations of *TLR10* and *IRAK2* with the risk of HD in Han Chinese individuals. These genetic etiology and pathogenesis data provide clues for understanding the association between *TLR10*/*IRAK2* and HD, and this research will ensure reductions in HD-related morbidity and mortality.

Materials and methods

Study subjects

In the present study, a total of 973 women with HD and 2681 healthy women, controls without any systematic disease, were recruited from the Second Affiliated Hospital of Xi'an Jiaotong University and the Northwest Women and Children Hospital between June 2012 and August 2017. All patients were diagnosed with HD based on an enlarged thyroid, characteristic ultrasound signs (hypoechogenicity and nonhomogeneous texture) and a high level of either antithyroid peroxidase or antithyroglobulin, with or without clinical and biochemical hypothyroidism. Those with a reported history of thyroid cancer and/or previous thyroid surgery were excluded from the study. The healthy controls had normal thyroid functions and no ultrasound changes in the thyroid, and they were negative for thyroid autoantibodies. Exclusion criteria included the existence of any comorbid cardiac, autoimmune, infectious, musculoskeletal or malignant disease, or a recent history of operation or trauma. All participants were unrelated Han Chinese individuals, and the case and control groups were matched by age. The Roche Diagnostics Cobas 6000 E601 Module Immunochemistry Analyzer (Roche, Basal, Switzerland) was used for thyroid function tests, and self-administered questionnaires were used to collect demographic data. The characteristics of our study subjects are shown in Table 1. No significant differences were observed in age, smoking status, or drinking status between the HD cases and healthy controls. Significant differences were found in family history and several clinical parameters (Table 1). The study protocol was approved by the Medical Ethics Committee of Xi'an Jiaotong University in accordance with the ethical guidelines of the Declaration of Helsinki of 1975 (revised in 2008). Written informed consent was obtained from the participants.

SNP selection and genotyping

Candidate SNPs were selected based on 1000 Genomes Chinese Han Beijing population (CHB) data. We searched for all SNPs with a minor allele frequency (MAF) ≥ 0.05 within the regions of the *IRAK2* and *TLR10* genes. Then, MAF ≥ 0.05 together with $r^2 \geq 0.5$ for *IRAK2* and $r^2 \geq 0.7$ for *TLR10* were used as cutoff criteria during tag SNP selection, which generated 17 and 16 tag SNPs within the *IRAK2* and *TLR10* genes, respectively. General information about these 33 selected SNPs is summarized in Supplemental Table S1. Genomic DNA was extracted from peripheral blood leukocytes according to the manufacturer's protocol (Genomic DNA Kit, Axygen Scientific Inc., California, USA). Genotyping was performed for all SNPs using the

Table 1 Baseline characteristics and clinical parameters of patients and controls

	Patients ($n = 973$)	Controls ($n = 2681$)	Statistics	P
Mean age (years)	40.7 ± 6.3	40.5 ± 6.4	$T = 0.54$	0.59
Thyroid size				
Normal	61 (6%)	2681 (100%)		
I degree	148 (15%)	–		
II degree	697 (72%)	–		
III degree	67 (7%)	–	–	–
Smoking status				
No	919 (94)	2553 (95)		
Yes	54 (6)	128 (5)	$\chi^2 = 0.75$	0.39
Drinking status				
No	891 (92)	2464 (92)		
Yes	82 (8)	217 (8)	$\chi^2 = 0.07$	0.80
Family history				
Positive	123 (13)	86 (3)		
Negative	850 (87)	2595 (97)	$\chi^2 = 116.07$	$<2.2 \times 10^{-16}$
Clinical parameters				
FT_3 (pmol/L)	4.15 ± 0.76	3.36 ± 0.33	$T = 31.27$	$<2.2 \times 10^{-16}$
FT_4 (pmol/L)	15.36 ± 2.36	13.45 ± 1.30	$T = 24.00$	$<2.2 \times 10^{-16}$
TSH (mIU/L)	4.81 ± 0.64	1.70 ± 0.94	$T = 115.28$	$<2.2 \times 10^{-16}$
Anti-TPO (IU/mL)	503.12 ± 268.24	10.98 ± 7.88	$T = -57.22$	$<2.2 \times 10^{-16}$
Anti-Tg (IU/mL)	459.37 ± 254.29	28.79 ± 22.21	$T = -52.75$	$<2.2 \times 10^{-16}$

Data are expressed as mean ± SD. The Roche Diagnostics COBAS 6000 E601 Module Immunochemistry Analyzer was applied for thyroid function tests

Abbreviations and normal reference ranges of each parameter: FT_3 (free T_3, normal reference range: 3.1–6.8 pmol/L), FT_4 (free T_4, normal reference range: 12–22 pmol/L), TSH (thyroid stimulating hormone, normal reference range: 0.27–4.2 mIU/mL); Anti-TPO (anti-thyroid-peroxidase, normal reference range: 0–34 IU/mL); Anti-Tg (anti-thyroglobulin, normal reference range: 0–115 IU/mL)

Sequenom MassARRAY RS1000 system (Sequenom, San Diego, California, USA). The results were processed using Typer Analyser software, and genotype data were generated from the samples [25]. Case and control status were blinded during all genotyping processes for quality control [26]. Five percent of the samples were randomly repeated for genotyping, and the results were the same as before.

Statistical analyses

χ^2 tests were performed for each marker to evaluate the genetic risk of HD contribution by single markers. Linkage disequilibrium (LD) blocks were constructed and haplotype-based association analyses were performed by Plink [27]. Potential epistatic effects were evaluated by case-only analyses [28]. To minimize the effects of multiple comparisons, we analyzed only those SNP pairs that included at least one significant hit in the single-marker-based analyses. In addition, we conducted expression quantitative trait loci (eQTL) analyses on those SNPs significantly associated with HD. eQTL data from multiple human tissues were extracted from the GTEx database [29]. In general, Bonferroni corrections were applied to correct for multiple comparisons. For the single-marker-based analyses, the P value threshold used was $0.05/33 \approx 0.0015$. In addition, we also examined the distributions of some clinical indicators of thyroid hormonal status and antithyroid antibodies in patients (free T_3, free T_4, TSH, anti-TPO and anti-TG) with HD in relation to the significant SNPs. Analysis of variance (ANOVA) was performed to test the significance of the differences in these clinical indicators among the different genotypes. To investigate the potential functional consequences of the two significant SNPs, we utilized bioinformatics tool RegulomeDB (http://www.regulomedb.org/) [30]. RegulomeDB is a database annotating SNPs with known and predicted regulatory elements information integrated from GEO, the ENCODE project, and published literature.

Results

All our candidate SNPs were in Hardy-Weinberg equilibrium in the control samples (Supplemental Table S2). Two

Table 2 Significant SNPs identified from single marker based association analyses

CHR	LOCI	SNP	POS	A1	MAF Cases	MAF Controls	χ^2	*P*	OR [95% CI]	SE
3	*IRAK2*	rs165501	10209243	C	0.37	0.33	11.20	0.0008	1.20 [1.08–1.34]	0.06
4	*TLR10*	rs10004195	38784724	T	0.44	0.39	15.02	0.0001	1.23 [1.11–1.37]	0.05

CHR chromosome, *POS* position, *A1* tested allele/minor allele, *SE* standard error

Table 3 Results of haplotype-based association analyses

Loci	SNPs	χ^2	DF	*P*
IRAK2	rs776805-rs696356	0.90	2	0.64
IRAK2	rs263413-rs144272025	0.50	2	0.78
IRAK2	rs779909-rs12638403	0.29	2	0.86
TLR10	rs190006616-rs12512137-rs4129009	6.80	3	0.08
TLR10	rs11466655-rs11096956	0.44	2	0.80
TLR10	rs10856838-rs11466643	3.22	2	0.20
TLR10	rs79030744-rs10004195-rs568924323	66.88	3	1.99×10^{-14}

DF degree of freedom

SNPs, rs165501 (OR = 1.20, $P = 0.0008$, *IRAK2*) and rs10004195 (OR = 1.23, $P = 0.0001$, *TLR10*), survived after Bonferroni correction in the single-marker-based association analyses (Table 2 and Supplemental Table S3). Seven LD blocks were constructed (3 for *IRAK2* and 4 for *TLR10*, Supplemental Figs. S1 and S2). A 3-SNP haplotype of *TLR10*, rs79030744-rs10004195-rs568924323, was identified to be significantly associated with HD disease status ($\chi^2 = 66.88$, $P = 1.99 \times 10^{-14}$, Table 3 and Supplemental Table S4, *P* value threshold was 0.007). A total of 32 SNP pairs including either rs165501 or rs10004195 between *IRAK2* and *TLR10* were tested for potential epistatic effects. Although several SNP pairs were identified to be nominally significant, none of them survived after Bonferroni correction (Supplemental Table S5, *P* value threshold was 0.002).

We examined the distributions of the clinical indicators of thyroid hormonal status and antithyroid antibodies in HD patients in relation to rs165501 and rs10004195. As shown in Table 4, the most significant result was obtained for free T_4 and rs10004195 in HD patients ($P = 0.03$). However, this level of significance could not survive after multiple comparison correction. Thus, no significant findings were identified for clinical indicators in HD patients in relation to the two significant SNPs.

Based on gene expression data extracted from GTEx, both rs165501 and rs10004195 were identified to be eQTLs in specific human tissues (Supplemental Tables S6 and S7, threshold of *P* values was 5×10^{-4}). Rs10004195 was found to be significantly associated with the gene expression of *TLR10* in human pituitary tissues ($P = 2.00 \times 10^{-4}$), while rs165501 was significantly associated with the expression of *IRAK2* in human thyroid tissues ($P = 3.10 \times 10^{-6}$). Specifically, the C allele of rs165501 was significantly associated with an elevated gene expression level of *IRAK2* (Fig. 1). We have also examined the eQTL signals for rs10004195 which achieved genome-wide significance, and the results were summarized in Supplemental Table S8. Rs10004195 was also associated with the expression of some other TLRs including *TLR1* and *TLR6*, in the GTEx database. In the present study, although we have applied SNP-gene mapping strategy based on physical location, SNP rs10004195 was also functional related to some other genes located within ±1 Mb window. RegulomeDB has its own score system and a lower score for a SNP often indicates more functional significance. No results were obtained for rs165501. For rs10004195, we get the score of 1f. This SNP located at two important functional regions: DNaseI Hypersensitivity region and H3K27Ac mark region.

Discussion

In this study, we identified significant association signals between our candidate loci, *IRAK2/TLR10*, and HD disease status. The SNP rs10004195 in *TLR10* was identified to be significantly associated with HD, and the odds of A allele in HD patients was averagely 23% higher compared to the controls in our Chinese sample. This SNP was reported to be associated with AITD in a study of Korean children (including 85 AITD patients and 279 healthy controls) conducted by Cho et al. [16]. The A allele of rs10004195 was also reported as a risk allele but with a much larger OR (2.8) in this previous study than in ours. This difference might be partly due to the difference in the sample sizes of the two studies. With a much larger sample size, our estimation for the OR of the risk allele was more accurate with less uncertainty. In addition, to the best of our knowledge, an association signal of rs165501 in *IRAK2* with HD has never been reported before. A previous study identified a significant association between *IRAK1* and AITD [24], but the association of *IRAK2* and HD has never previously been reported in any population-based study. In our study, the odds of C allele of rs165501 was averagely 20% higher in HD patients than in controls. Replication studies based on other populations are needed in the future to validate our

Table 4 Thyroid hormonal status and anti-thyroid antibodies in patients with HD in relation to the significant polymorphisms [mean (range)]

Loci	SNP	Genotypes ($N=973$)			ANOVA	
IRAK2	rs165501	CC ($N=138$)	CT ($N=438$)	TT ($N=397$)	*F*	*P*
	Free T_3 (pmol/L)	4.11 (3.17–5.88)	4.13 (3.17–5.89)	4.18 (3.17–5.89)	1.17	0.28
	Free T_4 (pmol/L)	14.91 (12.57–21.36)	15.44 (12.54–21.45)	15.45 (12.54–21.41)	3.50	0.06
	TSH (IU/mL)	4.81 (4.28–7.09)	4.79 (4.28–7.08)	4.83 (4.28–7.08)	0.51	0.48
	Anti-TOP (IU/ml)	520.70 (72.10–983.31)	492.18 (73.31–1008.21)	509.08 (70.87–1007.32)	1.00×10^{-4}	0.99
	Anti-TG (IU/ml)	440.00 (152.92–1068.96)	451.62 (151.52–1100.19)	474.67 (151.69–1107.32)	2.54	0.11
TLR10	rs10004195	TT ($N=190$)	TA ($N=469$)	AA ($N=314$)	*F*	*P*
	Free T_3 (pmol/L)	4.13 (3.17–5.80)	4.15 (3.17–5.89)	4.15 (3.17–5.89)	0.09	0.77
	Free T_4 (pmol/L)	15.43 (12.54–21.41)	15.55 (12.54–21.45)	15.05 (12.54–21.36)	4.61	0.03
	TSH (IU/ml)	4.87 (4.28–7.03)	4.78 (4.28–7.09)	4.81 (4.28–7.07)	0.71	0.40
	Anti-TOP (IU/ml)	538.51 (90.92–1006.19)	494.86 (74.15–1008.21)	494.05 (70.87–1008.11)	2.61	0.11
	Anti-TG (IU/ml)	437.26 (152.06–1100.19)	466.02 (152.92–1107.32)	462.83 (151.52–1103.08)	0.89	0.35

TSH thyroid stimulating hormone, *Anti-TPO* anti-thyroid-peroxidase, *Anti-Tg* anti-thyroglobulin, *ANOVA* analysis of variance

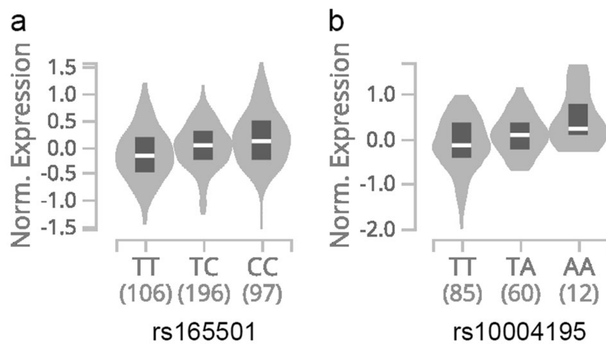

Fig. 1 Gene expression levels of *IRAK2* and *TLR10* for different genotypes of significant SNPs. **a** Gene expression levels of *IRAK2* for different genotypes of rs165501 in human thyroid tissue. **b** Gene expression levels of *TLR10* for different genotypes of rs10004195 in human pituitary tissue

findings of the association between *IRAK2* and HD or AITD.

Combined with eQTL data extracted from the GTEx database, we explored the potential effects of SNPs that were significantly associated with HD disease status on gene expression levels. Both significant hits showed significant eQTL signals in specific human tissues, and the eQTL pattern of rs165501 was particularly interesting. Our data showed that this SNP could significantly affect the gene expression of *IRAK2* in human thyroid tissue, which is a tissue directly related to HD and AITD. The C allele of this SNP seemed to be related to elevated *IRAK2* expression. Notably, this C allele is also the risk allele for HD status. Early studies have shown that downregulated expression of *IRAK2* could inactivate the NF-κB system [31]. A previous study has reported that HD with chronic inflammation causes a distinct increase in the levels of cytokines and other inflammatory mediators, such as IL-2, INF-gamma, IL-12 and IL-18 [32]. Activation of NF-κB induces the expression of immune response genes [33], including IL-12, which was induced in HD. Coincidentally, the involvement of NF-κB and IL-6 in HD can be seen in a recent study, which indicated that *NFKB1* was associated with HD through modulating IL-6 serum levels [34]. Another study demonstrated that the expression of NF-κB and IL-6 was upregulated in the thyroid tissues of adults with HD [35]. Thus, the participation of NF-κB in HD can be confirmed. Interestingly, a genetic variant of *IRAK2* can increases NF-κB activity through promoting TRAF6 ubiquitination, and the downstream regulators of NF-κB and IL-6 were excessively activated when IRAK2 was overexpressed [36]. In HD patients, a significantly increased prevalence of variants in the *IRAK1-MECP2* region was found [24]. Moreover, our findings on the genetic risk of rs165501 for HD and its eQTL features indicated that this SNP might affect the onset of HD, and this effect was mediated by its effect on the gene expression of *IRAK2*. Considering the abovementioned information, it is reasonable to hypothesize that *IRAK2* plays an essential role in HD through regulating NF-κB and its downstream targets, IL-6 and IL-12. Therefore, a better understanding of the role of *IRAK2* in the development of HD may allow early identification of individuals at risk and may even establish novel therapeutic targets in the future.

The evidence has shown a potential biological and functional link between the proteins encoded by *IRAK2* and *TLR10* [23, 24]. Therefore, we hypothesized that this biological link might be reflected at the level of DNA variations. We conducted a case-only analysis to model the potential gene-by-gene interaction between *IRAK2* and *TLR10*. SNPs from these two loci were expected to be

significantly correlated with each other in the case-only samples when epistasis was present. However, no significant signals were obtained from our analysis. This negative result might be partly due to a lack of statistical power, since a larger sample size is often required for gene-by-gene interaction analysis than for single-marker-based association analysis. More research is still needed in the future to detect potential gene-by-gene interactions between *IRAK2* and *TLR10*.

Our study suffered from several limitations. First, in this study, we included only women as our study subjects. Although this sample selection strategy can be partly justified by the higher prevalence of HD in women, it still produced a potential obstacle to generalization of the study results. Another potential limitation of this study is potential false positive signals due to population stratification. Although it is difficult to draw convincing conclusions only from SNP-based association analysis [37–44], haplotype-based association analyses have validated our findings based on single-marker association analyses. However, as one of the major confounders of genetic epidemiology studies, population stratification might severely affect the study results and cause false positive findings. Thus, the results might need to be applied with caution, especially because the Chinese population contains a large amount of genetic heterogeneity. Nevertheless, in this candidate-gene-based study, we could not perform some of the statistical methods that can be applied to genome-wide association studies, such as genomic control or principle component analyses. However, in our participant recruitment process, we tried to restrict the genetic background of our study subjects by applying some selection criteria, such as filtering out those subjects with a familial migration history within three generations. We believe that this strategy at least partly addressed some of the confounding effects of population stratification.

In conclusion, we identified significant association signals between HD disease status and two relevant loci, *IRAK2* and *TLR10*, in the Han Chinese population. Our findings suggested that both *IRAK2* and *TLR10* play important roles in the onset and development of HD. Given the complex pattern of HD etiology, its potential underlying genetic heterogeneity and its mechanism of chronic inflammation, wider replications in different ethnic samples and further functional analysis are warranted in the pursuit of comprehensive knowledge of the contributions of *IRAK2* and *TLR10* to the development of HD.

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Compliance with ethical standards

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

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