Original Article

Association between Polymorphism of Interleukin-23 Receptor and Hashimoto's Thyroiditis in Chinese Han Population of Shandong

Zhi-Hua Li[1], Jie Han[2], Yu-Fei Wang[2], Jun Dai[1], Hui Zhang[1], Chun-Xia Li[1], Qun Ma[1]

[1]Academy of Basic Medicine, Jining Medical University, Jining, Shandong 272067, China

[2]Department of Surgery, The Affiliated Hospital of Jining Medical University, First People's Hospital of Jining, Jining, Shandong 272011, China

Abstract

Objective: The interleukin-23 receptor (IL-23R) has been shown to be associated with autoimmune diseases in many different populations. This study aimed to investigate the association between *IL-23R* gene polymorphism and susceptibility to Hashimoto's thyroiditis (HT) in Chinese Han population of Shandong.

Methods: A case–control cohort study was performed in 145 HT patients from First People's Hospital of Jining between February 2010 to October 2013 and 150 healthy controls. Two single nucleotide polymorphisms located in the promoter region of *IL-23R* gene (rs17375018 and rs7517847) were examined by polymerase chain reaction-restriction fragment length polymorphism analysis. Hardy-Weinberg equilibrium was performed using the Chi-square test. Genotype frequencies were estimated by direct counting, and allele and genotype frequencies between patients and controls were analyzed by the Chi-square test.

Results: The rs17375018 GG genotype and the G allele were significantly increased in HT patients compared with healthy controls ($P = 0.034$ and $P = 0.013$, respectively). No association was identified between HT patients and healthy controls in rs7517847.

Conclusion: The study demonstrated that polymorphism of *IL-23R* gene rs17375018 is highly associated with HT in Chinese Han population of Shandong, suggesting that *IL-23R* gene polymorphism (rs17375018 G) may play a critical role in susceptibility to HT.

Key words: Genotype; Hashimoto's Thyroiditis; Interleukin-23 Receptor; Polymorphism

Introduction

Hashimoto's thyroiditis (HT), also called autoimmune thyroiditis, is an autoimmune disease, and clinically, it is the most common thyroid-related inflammation. Genetic factors, especially the susceptibility genes, play an important role in the pathogenesis of this disease. Many immune and proinflammatory mediators have been identified to be associated with susceptibility to HT. Among them, interleukin-23 receptor (*IL-23R*) gene has been shown to be associated with various autoimmune diseases and regulate the expression of genes involved in inflammation. The *IL-23R* gene is located on human chromosome 1p31.3, and its open reading frame is composed of 12 exons with total length around 92 kb. The single nucleotide polymorphisms (SNPs) sites of *IL-23R* are commonly located on the promoter, exon, or intron regions of the *IL-23R* gene. IL-23 is generally expressed on the cell surface of T lymphocytes, natural killer cells, and dendritic cells.[1,2] IL-23R, a member of hemopoietin receptor superfamily, is the specific component of the heterodimeric receptor complex for IL-23 signaling cascade. Therefore, IL-23 and IL-23R signaling pathway plays a significant role in immune regulation. It has been demonstrated that *IL-23R* gene SNPs are strongly correlated with various chronic inflammatory diseases including Crohn's disease,[3] rheumatoid arthritis,[4] ankylosing spondylitis (AS),[5] and Behcet's disease (BD). However, the linkage between *IL-23R* SNPs and susceptibility to HT has not been well-established. In the present study, we aimed to elucidate whether the polymorphisms of the *IL-23R* gene contribute to the development of HT in Chinese Han population of Shandong by analyzing two SNPs rs17375018 and rs7517847 of *IL-23R* gene, and highlight the potential role of *IL-23R* polymorphisms in the pathogenesis of HT.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.161362

Methods

Study population

A total of 145 HT patients from First People's Hospital of Jining between February 2010 to October 2013 and 150

Address for correspondence: Dr. Jie Han, Department of Surgery, The Affiliated Hospital of Jining Medical University, First People's Hospital of Jining, Jining, Shandong 272011, China
E-Mail: hanj3669@163.com

healthy controls were recruited in this case–control cohort study. All patients were of Chinese Han ethnic origin, and the control population consisted of unrelated healthy individuals, they were age-, sex-, and ethnicity-matched with the patients. All the study participants provided written informed consent, and the study protocols were approved by Ethical Committee of First People's Hospital of Jining. The HT patients were diagnosed according to the documented clinical and biochemical hypothyroidism requiring thyroid hormone replacement therapy and exhibited autoantibodies against thyroid peroxidase with or without antibodies against thyroglobulin (TG).[6] The clinical characteristics of the HT patients were recorded at the time of diagnosis. Clinical features and demographic characteristics of two groups were summarized in Table 1.

Single nucleotide polymorphism selection and genotyping

The rs17375018 and rs7517847 were selected as candidate SNPs, which were demonstrated earlier by another group of researchers to be associated with certain immune-related diseases.[7] A 5-μl reaction mixture, which consisted of 2.5 μl Premix Taq, 20 pmol primers, and 0.2 μg of genomic deoxyribonucleic acid, was amplified by polymerase chain reaction (PCR). The primers used in this study were presented in Table 2. The conditions were as follows: Initial denaturation at 95°C for 5 min, followed by 38 cycles of denaturation at 94°C for 30 s, annealing at different temperatures (55°C for rs17375018, and 58°C for rs7517847) for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. These SNPs were genotyped by PCR-restriction fragment length polymorphism analysis. The PCR products of the rs17375018 and rs7517847 polymorphisms were digested overnight with BsurI (New England Biolabs, Inc., Ontario, Canada) and Ec0147I (New England Biolabs, Inc., Ontario, Canada) restriction enzymes [Table 2] in a 10-μl reaction volume. Digestion products were visualized on a 3.5% agarose gel and stained with Gold View (SBS Genetech, Beijing, China) [Figure 1]. Direct sequencing was performed by Invitrogen Biotechnology Company (Guangdong, China) using randomly selected subjects (20% of all samples) to validate the method used in this study. Results were completely consistent.

Statistical analysis

Hardy-Weinberg equilibrium was performed using Chi-square test. Genotype frequencies were estimated by direct counting. Allele and genotype frequencies were compared between patients and healthy controls by the

Table 1: Clinical features and demographic characteristics of the study population

Characteristics	HT group (n = 145)	Control group (n = 150)
Male/female, n	11/134	15/135
Age, years, mean (range)	38.02 (15–67)	42.19 (18–63)
TSH, mIU/L, mean ± SD	53.60 ± 30.94	2.01 ± 0.78
FT_4, pmol/L, mean ± SD	2.85 ± 0.56	3.46 ± 0.64
FT_3, pmol/L, mean ± SD	0.64 ± 0.06	0.95 ± 0.08
Anti-TG, U/ml, mean ± SD	931.46 ± 540.22	<115
Anti-TPO, U/ml, mean ± SD	401.62 ± 229.56	<10
TRAb, ng/L, mean ± SD	13.2 ± 3.77	12.1 ± 1.15

TSH: Thyroid stimulating hormone; FT_4: Free thyroxine; FT_3: Free triiodothyronine; Anti-TG: Anti-thyroglobulin autoantibodies; Anti-TPO: Anti-thyroid peroxidase antibodies; TRAb: Thyrotrophin receptor antibody; SD: Standard deviation.

Table 2: Primers and restriction enzymes used for RFLP analysis of *IL-23R* gene

SNPs	Primers	Restriction enzyme
rs17375018	5'-TTTTTCCCATCT TCTTTCTTAA-3'(forward)	BsurI
	5'-CGCCCAGCCCT CTTCTCTAATT-3'(reverse)	
rs7517847	5'-CCTTTCACCTAT TCCCAAGGCC-3' (forward)	Ec0147I
	5'-GGGCCTAGGAG ACAGCCCATAA-3' (reverse)	

SNPs: Single nucleotide polymorphisms; RFLP: Restriction fragment length polymorphism.

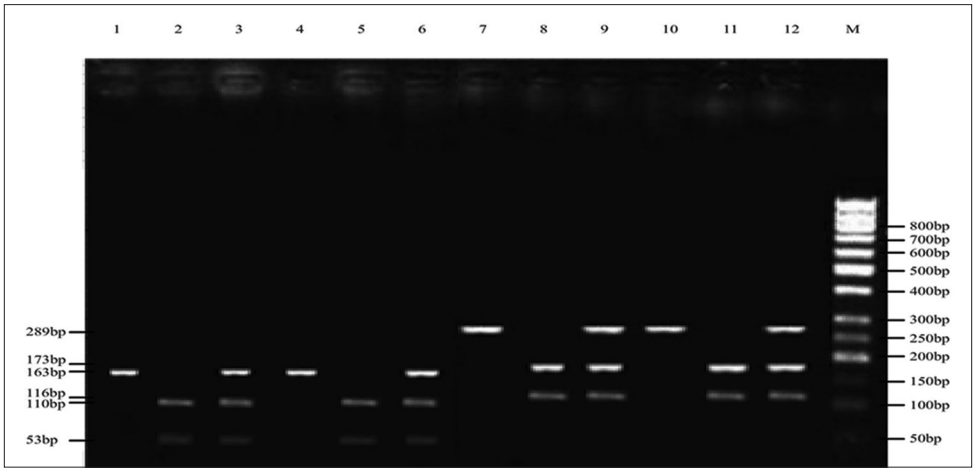

Figure 1: Restriction enzyme digestion results of rs17375018 and rs7517847. M: Generuler 50 bp deoxyribonucleic acid ladder; Lane 1, 4: rs7517847 TT genotype; Lane 2, 5: rs7517847 GG genotype; Lane 3, 6: rs7517847 TG genotype; Lane 7, 10: rs7375018 GG genotype; Lane 8, 11: rs7375018 AA genotype; Lane 9, 12: rs7375018 GA genotype.

Table 3: Hardy–Weinberg genetic equilibrium test results of rs17375018 and rs7517847

SNPs	Genotype allele	HT group			Control group		
		Observed number	Theoretical value	*P*	Observed number	Theoretical value	*P*
rs17375018	AA	6	7.51	0.476	16	15.36	0.810
	GA	54	50.97		64	65.28	
	GG	85	86.51		70	69.36	
rs7517847	TT	51	51.60	0.836	53	54.60	0.585
	GT	71	69.79		75	71.79	
	GG	23	23.60		22	23.60	

HT: Hashimoto's thyroiditis; SNPs: Single nucleotide polymorphisms.

Table 4: Frequencies of alleles and genotypes of *IL-23R* polymorphisms in HT patients and healthy controls

SNPs	Genotype Allele	HT group, *n* (%) (*n* = 145)	Control group, *n* (%) (*n* = 150)	χ^2	*P*	*OR* (95% *CI*)
rs17375018	AA	6 (4.2)	16 (10.7)			0.626 (0.434–0.903)
	GA	54 (37.2)	64 (42.7)			
	GG	85 (58.6)	70 (46.6)	6.762	0.034	
	A	66 (22.8)	96 (32.0)			
	G	224 (77.2)	204 (68.0)	6.322	0.013	
rs7517847	TT	51 (35.2)	53 (35.3)			1.029 (0.740–1.430)
	GT	71 (49.0)	75 (50.0)	0.086	0.958	
	GG	23 (15.8)	22 (14.7)	0.028	0.867	
	T	173 (59.7)	181 (60.3)			
	G	117 (40.3)	119 (39.7)			

HT: Hashimoto's thyroiditis; SNPs: Single nucleotide polymorphisms; *OR*: Odds ratio; *CI*: Confidence interval.

Chi-square test. All normally distributed data were shown as mean ± standard deviation (SD). All data analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

Results

Hardy-Weinberg equilibrium analysis was performed for two *IL-23R* genetic variants in the HT and control groups. Results of Hardy–Weinberg genetic equilibrium test were shown in Table 3. The distribution of both genotypic frequencies and allelic frequencies of the two tested IL-23 polymorphisms was shown in Table 4. The results showed that there were significant differences between patients with HT and controls concerning the frequencies of rs17375018. The frequencies of the rs17375018 GG genotype and G allele in patients with HT were significantly increased compared with healthy controls ($P = 0.034$ and $P = 0.013$, respectively). There was no difference concerning the frequencies of the rs7517847 SNP between the HT and control groups.

Discussion

Hashimoto's thyroiditis is a common autoimmune inflammatory disease, and TG and thyroid peroxidase antibody has been detected in HT patients. Pathology of this disease mainly includes atrophy and damage of thyroid follicular epithelial cells. Previous studies have shown that IL-17 secreted by Th17 cells is involved in the inflammatory damage, and IL-17 production is regulated by many cytokines including IL-6 and IL-23.[8,9] It is known that IL-23 can promote inflammation by regulating various proinflammatory cytokines including IL-17, IL-6, IL-8, and tumor necrosis factor (TNF)-α, and maintain the amplification and stability of the Th17 cells.[10] Thus, we hypothesized that the IL-23-IL-23R signaling pathway may contribute to the pathogenesis of HT. The interaction of IL-23R with its ligand, IL-23, can promote the differentiation of IL-17, which is known to be involved in many chronic inflammatory diseases.[11] Therefore, IL-23R is critical in the control of the biological activity of IL-23. So far, the association between IL-23R and susceptibility to HT has not been clearly established. The present study demonstrated that only rs17375018 was associated with the risk of HT. The IL-23R rs17375018 allele frequency of G in HT group was significantly higher than that in the healthy control group, suggesting that the GG genotype might be a susceptible gene locus for HT by acting its effect on target gene transcription. Some similar studies in Japanese[12] and Croatian population[13] have reported the different conclusions, which suggested the racial differences in the relationship between gene polymorphism and diseases.

It is known that IL-23R is a type I transmembrane protein and can lead to activation of downstream molecules Jak2 and Tyk2 when its extracellular region is combined with IL-23. Two signal transducers and activators of transcription (STAT) can generate a dimer, and the phosphorylated STATs dimer can move into the nucleus to target gene activation.[14] SNPs in the *IL-23R* gene cause changes in messenger ribonucleic acid (mRNA) splicing factor and coded amino acid sequence,

thus affecting the gene expression and protein function. The rs17375018 is located in the inner segment of *IL-23R* gene. It can be speculated that the rs17375018 G allele can effectively activate their downstream molecules leading to the activations of target genes in the pathogenesis of HT. Thus, activation of IL-23R can promote the differentiation of Th1 cells into Th17 cells subgroup;[15] and it can increase the release of IL-17, TNF-α, and inflammatory factors, thus to participate in the chronic inflammatory process of HT. Interestingly, Jiang *et al*.[7] reported that a potential transcription factor binding site for sp-1 existed in the rs17375018 G allele, but not in the rs17375018 A allele. It has been shown that sp-1 can suppress the expression of IL-10, resulting in the enhancement of inflammation.[16] However, some studies showed an increased level of IL-10 in patients with BD.[17,18] These discrepant results may partly be explained by the fact that the IL-10 serum level is profoundly influenced by disease activity and medicines used for treatment. Future studies are needed to validate the molecular mechanisms involved in HT.

Recently, some studies showed the associations between rs7517847 gene polymorphism and inflammatory bowel disease, allergic rhinitis,[19] AS,[20] and other autoimmune diseases. These studies suggested that the intronic polymorphism rs7517847 might exert its influence by regulating the specific splicing of the gene.[21] The rs7517847 T → G change may affect mRNA length and protein product.[22] However, our study indicated that the polymorphism of rs7517847 had no significant difference between the HT and control groups. This might be related to differences in sample population, ethnicity, geography, and sample size. Thus, future study with a larger sample size is need to further validate this relationship.

In conclusion, this study demonstrated that polymorphism of *IL-23R* gene rs17375018 is highly associated with HT in Chinese Han population of Shandong, suggesting that *IL-23R* gene polymorphism (rs17375018 G) may play a critical role in susceptibility to HT. The GG genotype and G allele of rs17375018 were predisposing factors for HT. The G allele of rs17375018 may be a potential therapeutic target for HT in Chinese Han population. In the future, the biological function of rs17375018 needs to be further explored, it is necessary to clarify whether the loci associated with HT identified in the present study and other studies are only markers or causative variants.

References

1. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, *et al.* A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J Immunol 2002;168:5699-708.
2. Trinchieri G, Pflanz S, Kastelein RA. The IL-12 family of heterodimeric cytokines: New players in the regulation of T cell responses. Immunity 2003;19:641-4.
3. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, *et al.* A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science 2006;314:1461-3.
4. Hollis-Moffatt JE, Merriman ME, Rodger RA, Rowley KA, Chapman PT, Dalbeth N, *et al.* Evidence for association of an interleukin 23 receptor variant independent of the R381Q variant with rheumatoid arthritis. Ann Rheum Dis 2009;68:1340-4.
5. Dong H, Li Q, Zhang Y, Tan W, Jiang Z. IL23R gene confers susceptibility to ankylosing spondylitis concomitant with uveitis in a Han Chinese population. PLoS One 2013;8:e67505.
6. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: Clinical and diagnostic criteria. Autoimmun Rev 2014;13:391-7.
7. Jiang Z, Yang P, Hou S, Du L, Xie L, Zhou H, *et al.* IL-23R gene confers susceptibility to Behcet's disease in a Chinese Han population. Ann Rheum Dis 2010;69:1325-8.
8. Figueroa-Vega N, Alfonso-Pérez M, Benedicto I, Sánchez-Madrid F, González-Amaro R, Marazuela M. Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. J Clin Endocrinol Metab 2010;95:953-62.
9. Torchinsky MB, Blander JM. T helper 17 cells: Discovery, function, and physiological trigger. Cell Mol Life Sci 2010;67:1407-21.
10. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, *et al.* The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells *in vivo*. Nat Immunol 2009;10:314-24.
11. Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol 2007;8:345-50.
12. Ban Y, Tozaki T, Taniyama M, Nakano Y, Yoneyama K, Ban Y, *et al.* Association studies of the IL-23R gene in autoimmune thyroid disease in the Japanese population. Autoimmunity 2009;42:126-30.
13. Stevic MS, Stefanic M, Tokic S, Glavas-Obrovac L, Mihaljevic S, Karner I. Pilot study of variants of the IL-23R and STAT3 genes reveals no association with Hashimoto's thyroiditis in the Croatian population. Endocr Res 2014;39:164-7.
14. Huber AK, Jacobson EM, Jazdzewski K, Concepcion ES, Tomer Y. Interleukin (IL)-23 receptor is a major susceptibility gene for Graves' ophthalmopathy: The IL-23/T-helper 17 axis extends to thyroid autoimmunity. J Clin Endocrinol Metab 2008;93:1077-81.
15. Ferguson LR, Han DY, Fraser AG, Huebner C, Lam WJ, Morgan AR. IL23R and IL12B SNPs and haplotypes strongly associate with Crohn's disease risk in a New Zealand Population. Gastroenterol Res Pract 2010;2010:539461.
16. Maroof A, Beattie L, Zubairi S, Svensson M, Stager S, Kaye PM. Posttranscriptional regulation of IL10 gene expression allows natural killer cells to express immunoregulatory function. Immunity. 2008;29:295-305.
17. Hamzaoui K, Hamzaoui A, Guemira F, Bessioud M, Hamza M, Ayed K. Cytokine profile in Behçet's disease patients. Relationship with disease activity. Scand J Rheumatol 2002;31:205-10.
18. Mantas C, Direskeneli H, Eksioglu-Demiralp E, Akoglu T. Serum levels of Th2 cytokines IL-4 and IL-10 in Behçet's disease. J Rheumatol 1999;26:510-2.
19. Hu D, Hu G, Zhu J, Shen Y, Kang H, Hong S. Association between polymorphisms of the IL-23R gene and allergic rhinitis in a Chinese Han population. PLoS One 2013;8:e63858.
20. Karaderi T, Harvey D, Farrar C, Appleton LH, Stone MA, Sturrock RD, *et al.* Association between the interleukin 23 receptor and ankylosing spondylitis is confirmed by a new UK case-control study and meta-analysis of published series. Rheumatology (Oxford) 2009;48:386-9.
21. Zhang XY, Zhang HJ, Zhang Y, Fu YJ, He J, Zhu LP, *et al.* Identification and expression analysis of alternatively spliced isoforms of human interleukin-23 receptor gene in normal lymphoid cells and selected tumor cells. Immunogenetics 2006;57:934-43.
22. Safrany E, Melegh B. Functional variants of the interleukin-23 receptor gene in non-gastrointestinal autoimmune diseases. Curr Med Chem 2009;16:3766-74.

Received: 27-02-2015 **Edited by:** Xin Chen
How to cite this article: Li ZH, Han J, Wang YF, Dai J, Zhang H, Li CX, Ma Q. Association between Polymorphism of Interleukin-23 Receptor and Hashimoto's Thyroiditis in Chinese Han Population of Shandong. Chin Med J 2015;128:2050-3.

Source of Support: The study was supported by a grant from the Research Project of the Education Department of Shandong Province (No. J12LK16). **Conflict of Interest:** None declared.