Association Between IL-18 Gene Polymorphism and Hashimoto Thyroiditis

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Research Article

Keywords: Hashimoto's thyroiditis, Interleukin 18 gene polymorphism, -137 IL18 CG genotype, -607 AC genotype

Posted Date: May 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-510648/v1

Abstract

Background

Hashimoto's thyroiditis (HT) is the most frequent organ-specific autoimmune disease (AIT), which is called lymphocytic thyroiditis in which T helper-1 lymphocytes mediate the disease. IL-18 is expressed in thyroid follicular cells (TFCs) during HT. The findings of studies aimed at investigating the relationship between IL-18 and HT are highly contradictory. In this study, we aimed to investigate the association between IL-18 gene polymorphism and HT.

Methods and Results

The study was included 97 patients diagnosed with HT and 86 volunteers in the healthy control group. The IL18-607C/A (rs1946518) ve -137G/C (rs187238) genotypes were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. No significant difference in the mean ages and gender was observed between the groups ($p = 0.763$ and $p = 0.658$, respectively). -137 IL18 CG genotype is higher in HT than controls. The risk of the IL-18 CG genotype for HT patients was more than 2.237 times higher (OR; 2.237 %95 CI: 1.195–4.187, $p = 0.039$) compared to that of the G/G genotype. Also, -607 AC genotype is higher in the control group than in the HT group (in individuals with the IL18 CG genotype).

Conclusions

According to our results, the CG genotype might be a risk factor for HT. Conversely, there is a possibility that the AC genotype also plays a protective role against the condition. However, further studies will contribute to the emergence of new solutions by revealing the molecular and cellular mechanisms of HT.

Introduction

Hashimoto's thyroiditis (HT) is the most frequent autoimmune disease of the thyroid gland. It accounts for 30% of organ-specific autoimmune diseases (AIT) [1]. HT is the most common cause of hypothyroidism in the iodine-sufficient areas of the world. About 20–30% of patients with HT suffer from hypothyroidism [2, 3]. According to the NHANES III study, the frequency of subclinical and clinical hypothyroidism in the USA was found to be 4.6% and 0.3%, respectively [4]. It is more common in women. The female to male ratio ranges from 5:1 to 10:1 [5].

The diagnosis of AIT depends on different characteristics: the presence of circulating antibodies against the thyroid gland; a hypoechogenic and dyshomogeneous gland parenchyma at ultrasonography; elevated levels of thyroid-stimulating hormone (TSH), with normal or low serum thyroid hormones (only in a fraction of patients) [2, 3].

HT is autoimmune thyroiditis and lymphocytic thyroiditis, an organ-specific complex disease in which T cell-mediated (especially T helper-1 lymphocyte) genetic factors play a role in the etiology for which the

immune regulatory system's defect is responsible [6].

IL-18, a member of the IL-1 superfamily, previously known as Interferon- γ (INF-γ) stimulating factor, stimulates the synthesis and gene expression of pro-inflammatory cytokines, CC/CXC chemokines, adhesion molecules, and at the same time activates many immune and non-immune cells such as T and B lymphocytes, natural killer cells, Langerhans cells, monocytes, neutrophils, macrophages and Kupffer cells [7, 8].

IL-18 is expressed in the inflammatory regions of various autoimmune diseases and many infectious diseases [8]. Up-regulation of IL-18 expression has been shown in animal models of Th-1-mediated autoimmune disorders such as Type 1 diabetes mellitus [9], Crohn's disease [10], lupus nephritis [11], multiple sclerosis [12], and Sjögren's syndrome [13].

It has been shown that IL-18 expressed in thyroid follicular cells (TFCs) in HT has a close relationship with lymphocyte infiltration [8] Li et al. observed that IL-18 m RNA levels with Reverse Transcription-polymerase chain reaction (RT-PCR) were significantly more expressed in HT patients than in controls. Also, they confirmed that with immunohistochemical staining in tissue was detectable more diffuse positive staining. They suggested that IL-18 plays a direct role in thyroid destruction in autoimmune thyroiditis [14].

Immunological factors have been considered to play crucial roles in HT's pathogenesis. The precise mechanisms by which such immunological factors contribute to HT's pathogenesis remain unknown.

The results of studies conducted so far on serum IL-18 levels and gene polymorphism in HT have been found to be quite contraversial [15–21].

This study aims at investigating IL-18 gene polymorphism in patients diagnosed with HT. We sought to assess the potential roles of IL18-137 and IL18-607 polymorphisms in patients with HT.

Material And Methods

The study was conducted on 97 patients who were admitted to the endocrinology outpatient clinic who was diagnosed with HT and 86 volunteers healthy control group. In addition to clinical findings of hypothyroidism, TSH, free thyroxine (fT4), anti-thyroidperoxidase-thyroidperoxidase (Anti-TPO), and anti-thyroglobulin-thyroglobulin (anti-Tg-Tg) antibodies were examined in the laboratory. Thyroid ultrasonography (US) was also performed on the patients.

Demographic data such as age and gender of the patients in the HT and control groups were recorded. Patients with chronic conditions such as heart failure, kidney failure, malignancy, smoking, and alcohol use were excluded from the study.

Genomic Dna Isolation And Genotype Analysis

The genomic DNA was isolated from peripheral blood lymphocytes by standard procedures using the Macherey-Napel Nucleospin blood® DNA extraction kit (Cat no. 740.951.250) according to the manufacturer's instructions. The IL18 -607C/A (rs1946518) ve -137G/C (rs187238) genotypes were determined through the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primer sequences (Karakas Celik S, Öz ZS, Dursun A, Unal A, Emre U, Cicek S, Keni FM). Interleukin 18 gene polymorphism is a risk factor for multiple sclerosis. MolBiol Rep. 2014 Mar;41(3):1653-8.), annealing temperatures and restriction enzyme are represented in Table 1. Amplified products were digested using 5 U of the Tru9I (rs1946518), 5 U of EcoRI (rs187238) restriction enzymes. Digested fragments were separated on 2% agarose gels through electrophoresis. Alleles were identified according to their fragment size (Table 2).

Table 1
Demographic characteristics and laboratory data of controls and patients with HT.

	Controls	Cases	P value
Total3	86 (100)	97 (100)	
Age. years Median (Min-Max)	37.27 ± 12.51	37.41 ± 11.34	0.763
Sex n (%)			0.658
Female	74 (86.0)	86 (88.7)	
Male	12 (14.0)	11(11.3)	
Anti-TPO (IU/mL) Median (Min-Max)	0.0 (0.00–802.00)	323.00 (0.00–1000.00)	0.001
Anti-TTG (IU/mL) Median (Min-Max)	0.0 (0.00–0.00)	69.7 (0.00–9100.00)	0.003
TSH (pg/mL) Median (Min-Max)	1.64 (0.26–4.70)	2.51 (0.10–100.0)	0.001
fT4 (pg/mL) Median (Min-Max)	0.82 (0.64–1.20)	0.82 (0.41–3.87)	0.940

Table 2
IL18 -607C/A (rs1946518) ve -137G/C (rs187238) single nucleotide polymorphisms (SNP), primer sequences, annealing temperatures, restriction enzymes, and allele sizes

SNP	Primer	Annealing Temp. (°C)	Restriction Enzymes	Allele Size (bp)
rs1946518	F;5'-GCC CTC TTA CCT GAA TTT TGG TAG CCC TC R; 5'-AGA TTT ACT TTT CAG TGG AAC AGG AGT CC 3')	60^0C	Tru9I	AA: 101, 70 AC: 171, 101, 70 CC: 171
rs187238	F; 5 ATG CTT CTA ATG GAC TAA GGA R; 5'-GTA ATA TCA CTA TTT TCA TGA ATT	50^0C	EcoRI	CC:131 GC:131, 107, 24 GG:107, 24

Statistical Analyses

Statistical analysis was performed using the SPSS software (version.19.0; SPSS Inc., Chicago, IL, USA). A case-control study was performed, and the allelic frequency of the polymorphism was calculated in both cases and controls. The ^{2} test was used to compare the allele frequency of each gene polymorphism between patients and controls. OR and 95% confidence interval (CI) were calculated to compare the HT risk around genotypes and alleles. P-values less than 0.05 were considered statistically significant.

Results

By using the “polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)” method in 97 patients diagnosed with Hashimoto Thyroiditis and 86 healthy controls; Single-nucleotide polymorphisms (SNPs) were studied.

No significant difference in the mean ages and gender ratios was observed between the groups (p = 0.763 and p = 0.658, respectively). There was a significant difference in terms of Anti-TPO-TPO, Anti-TTG-TTG, and TSH levels (p = 0.001; p = 0.003, p = 0.001), but we did not find any difference in terms of fT4 levels between the groups (p = 0.942). (Table 1)

The distributions of genotypes and allele frequencies of the IL18 -607C/A (rs1946518) ve -137G/C (rs187238) polymorphism in each group are shown in Table 3. The C/G genotype was more common among the cases than among the healthy controls and IL18 C/G genotypes were associated with increased risk for HT. The risk for HT was more than 2.237 times higher (OR; 2.237 %95 CI: 1.195–4.187,

p = 0.039) in individuals with the IL18 CG genotype than in those with the G/G genotype. Also, IL18-607 C/A genotype polymorphism was found to be more common in controls than in patients with HT. (OR = 0.295 95%-CI 0.117–0.745; p = 0.026). As a result, it was determined that the CA genotype might have a protective effect against HT.

Table 3
IL18-137 and IL18-607 genotypes and alleles in both groups

	Healthy Control n = 86 (%)	HT n = 97 (%)	p-value	‡ OR (95% CI)
IL18 -137 G/C Genotype frequency			0,039	
GG	48 (55,8)	37 (38,1)		Reference*
CG	29 (33,7)	50 (51,5)		2,237 (1,195–4,187)
CC	9 (10,5)	10 (10,3)		1,441 (0,532–3,908)
Allele frequency				
G	125 (72,7)	124 (63,9)	0,092	Reference
C	47 (27,3)	70 (36,1)		0,666 (0,427–1,040)
IL18 -607 C/A Genotype frequency				
AA	9 (10,5)	22 (22,7)	,026	Reference
CA	36 (41,9)	26 (26,8)		0,295 (0,117–0,745)
CC	41 (47,7)	49 (50,5)		0,489 (0,203–1,178)
Allele frequency				
	118 (68,6)	124 (63,9)	0,377	Reference
	54 (31,4)	70 (36,1)		0,811 (0,525–1,253)
ORs (odds ratio) ; CI (confidence interval) from conditional logistic regression.				
* Carriers of at least one intact allele are used as a reference.				

Haplotype frequency and bivariate analysis were also performed, but no statistically significant difference was noted (Table 4).

Table 4
Haplotypes frequencies of IL18-137/IL-607 polymorphisms in patients with HT and healthy controls.

Haplotype	Healthy Controls n = 86 (%)	HT Patients n = 97 (%)	p	OR ‡ (95% CI)
IL18 -137/ -607				
GC	100 (58,1)	98 (50,5)	0,352	Reference
GA	25 (14,5)	26 (13,4)		1,061 (0,573–1,964)
CC	18 (10,5)	26 (13,4)		1,474 (0,760–2,859)
AA	29 (16,9)	44 (22,7)		1,548 (0,897–2,671)

Discussion

The etiology of HT has still not been fully understood. It has been considered as a genetic predisposition triggered by environmental factors that cause the loss of immunological tolerance, which attack the TFCs and lead to chronic inflammation with lymphocytic infiltration, especially T helper cells, destruction, atrophy, and fibrosis of TFCs [2, 3, 9]. Although immunological factors have been considered to play crucial roles in the pathogenesis of HT, the precise mechanisms by which such immunological factors contribute to that pathogenesis remain unknown. In this study, we sought to assess the potential roles of polymorphisms of IL18-137 and IL18-607 in patients with HT.

Some studies reported that HT is a Th1-driven autoimmune disease. IFN-γ and IL-2 measured both serum and intrathyroidal lymphocytes in HT patients. As a result, activated T cells secrete cytokines such as Interferon γ (IFN-γ) and interleukin 2 (IL-2), and they contribute to Th1-mediated immune response to the destruction of TFCs [15].

In addition to that, activated helper T lymphocytes interact with B lymphocytes, and activated B lymphocytes form antibodies that react with thyroid antigens. These have a role in the apoptotic destruction of thyroid cells through the activation of cytotoxic T cells (a Th1 function) [22, 23].

Also, serum concentrations of IL-2, INF-γ, IL-12, and IL-18 were found to be significantly increased in HT patients than in the control group [15]. In another study, serum IL-18 levels in HT patients were not higher than those in controls, while patients with Grave's disease had higher IL-18 levels than controls [24]. We did not study IL-18 serum levels.

IL-18 was expressed in thyroid tissues of individuals with AIT by RT-PCR and immunohistology. It has been suggested that human TFCs increase IL-18 production, IL-18 together with IL-12, promotes INF-γ production in autoimmune thyroiditis. IFN-γ is essential for the development of lymphocytic autoimmune thyroiditis and the inhibition of thyrocyte proliferation [14]. Before Liu et al., Kaiser et al., 2002 demonstrated IL-18 expression in animal models in autoimmune TFCs [16].

Carrying C at position − 607 and G at position − 137 were shown to be associated with a significantly higher expression of the IL-18 protein because the − 607C allele and the 137 G allele have high promoter activity [17, 20].

Inoue et al. did not find any differences in genotype and allele of polymorphism in -607 A/C between HT and controls but the frequency of the − 607CC genotype is higher severe HT than in mild HT. However, we found the IL18-607 C/A genotype polymorphism more common in controls than in the HT group. They also did not find any significant differences in the − 137 GC genotype and allele [19]. Contrary to the findings of their study, we found that the − 137C/G polymorphism was more common among the cases than among the controls. This discrepancy might be due to ethnic differences.

Mukai et al. reported that they did not find any significant association between IL-18 polymorphism and AITD, especially Grave's disease [20]. Ide et al. studied IL-18 polymorphism (-137 (G/C) and − 607 (C/A) in AITD in people with and without diabetes. They found differences between polymorphism and AITD [21] but we found the − 137C/G polymorphism to be more common among the cases than among the controls. This difference in the results might be due to the increased risk for HT. Also, the IL18-607 C/A genotype polymorphism was more common in the control than in the patients in the HT group. The CA genotype might have a protective effect against HT.

Huang et al. conducted a study on 116 pediatric patients in which they identified an association between HT and IL-18 in Taiwan in 2012. They studied the same IL-18 polymorphism G/C genotype is higher in HT than controls similarly in our study. In contrast, the G/G genotype was less frequent in patients with HT than in controls. They suggested that the G/G genotype might be protective against HT. They did not find any differences in the − 607 T/G genotype and allele between the two groups. They assumed that the C allele and CT haplotype are risks for HT tendency [25], but we did not find any significant differences in the allele groups.

Our findings show that the − 137 CG genotype is more frequent in patients with HT than in controls. The risk for HT patients was more than 2.237 times higher in individuals with the IL18 CG genotype than in those with the GG genotype. Also, the − 607 AC genotype is higher in controls than in the HT group. So, we suggested that the AC genotype might be protective for HT. Therefore, it is reasonable to speculate that polymorphism in the IL-18 promoter could affect the balance between Th1 and Th2 cytokine responses. The deterioration in the balance might have contributed to the destruction of TFCs in individuals carrying these genotypes of IL-18, leading to a higher susceptibility to HT.

According to the assessments to be made based on this study's findings, the CG genotype might represent a risk factor for HT. Conversely, there is a possibility that the AC genotype also plays a protective role against HT. New developments in the science of genetics will contribute to the emergence of new solutions by revealing the molecular and cellular mechanisms of HT. Therefore, further studies that include environmental factors will contribute to the explanation of many factors that we do not know today.

Declarations

Authors' Contributions: Author contributions: Dilek karakaya and Sevim Karakas Celik designed the research. Dilek Karakaya and Tugba Aktas performed the research. Sevim Karakas Celik, Gunes Cakmak Genc and Tugba Aktas analysed the data. Taner Bayraktaroglu and Ahmet Dursun supervised the research. Dilek karakaya, Sevim Karakas Celik, Taner Bayraktaroglu and Ahmet Dursun wrote the manuscript.

Ethical approval: The research protocol was approved by the ethics committee of Zonguldak Bülent Ecevit university medical school on february 10, 2021 (Reference Number: 2021/03).

Data Avaibility: The dataset is available from the corresponding author on reasonable request.

Funding:none

Conflicts of interest: the authors declare that they have no conflict of interest.

Informed consent: Informed consent was obtained all participants.

Acknowledgements: Declared none.

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