Presence of more activating KIR genes is associated with Hashimoto’s thyroiditis

Elham Ashouri · Mohammad Hossein Dabbaghmanesh · Gholamhossein Ranjbar Omrani

Abstract Killer cell immunoglobulin-like receptors (KIR) regulate the effector function of natural killer (NK) cells and the subset of T cells with memory phenotype. The number and type of genes that encode KIR receptors substantially varied between individuals and between populations. Specific KIR receptors are known to be associated with certain diseases. The present study was undertaken to investigate if any specific KIR gene(s) is associated with the susceptibility to Hashimoto’s thyroiditis (HT), an inflammatory disease characterized by lymphocytic infiltration of the thyroid gland and the presence of autoantibodies directed against thyroglobulin and/or thyroid peroxidase. DNA from 118 patients with HT and 120 healthy controls was characterized for the presence and absence of 11 variable KIR genes using a gene-specific PCR typing system. Although no significant difference in the frequency of individual KIR genes between patients and controls was detected, more patients carry the six activating KIR genes compared with the control group (11.8 vs. 4.1 %,

\[ p = 0.032, \ OR = 3.09, \ 95 \% \ CI \ 1.07–8.89 \]). The data suggest that augmented signals from multiple activating KIR receptors might exacerbate the activation of NK cells and T cell subsets against self-antigens, thus contributing to the pathogenesis of HT.

Keywords KIR · Gene content profiles · PCR-SSP · Hashimoto’s thyroiditis

Introduction

Natural killer (NK) cells are crucial components of the innate immune system and provide a first line of defense against tumor transformation and infection [1]. In order to function correctly, NK cells must identify the infected cells without affecting normal healthy cells. For this reason, the inhibitory NK cell receptors engage human leukocyte antigen (HLA) class I on target cells. The extraordinarily diverse interactions between polymorphic HLA-A, -B, and -C molecules and the family of killer cell immunoglobulin-like receptors (KIR) play a role in expanding and individualizing the human immune systems [2–4]. The KIR gene family encodes 14 receptors; seven (KIR3DL1, 3DL2, 3DL3, 2DL1, 2DL2, 2DL3, and 2DL5) are involved in the inhibition of NK cell response, six (KIR3DS1, 2DS1, 2DS2, 2DS3, 2DS4, and 2DS5) are involved in the activation of NK cell response, and one (KIR2DL4) in mediating both inhibitory and activating functions [2, 5].

Inhibitory KIR receptors recognize distinct HLA class I molecules: KIR2DL2 and 2DL3 bind HLA-C allotypes containing an asparagine at amino acid position 80 (group C1 alleles); KIR2DL1 binds HLA-C allotypes with lysine at amino acid position 80 (group C2 alleles) preventing NK cells from attacking autologous cells [6, 7]; KIR3DL1 binds HLA-B allotypes containing the Bw4 epitope [8]; and KIR3DL2 binds HLA-A3/11 allotypes [9]. The ligands for activating KIRs have yet to be elucidated. Besides NK cells, KIR receptors are also expressed on subpopulations of \( \alpha \beta \) and \( \gamma \delta \) T cells, indicating a KIR receptor role in adaptive immunity [10].

KIRs are encoded by a compact cluster of genes on chromosome 19q13.4 [5, 11]. The combinations of variable gene content and allelic polymorphism diversify the KIR gene variation in humans [12, 13]. The number and type of KIR
genes vary substantially by haplotypes, and two groups of haplotypes have been characterized [5, 14]. Group A haplotypes contain a fixed number of KIR genes (KIR3DL3-2DL3-2DP1-2DL1-3DP1-2DL4-3DL1-2DS4-3DL2) including a single activating gene, KIR2DS4. Group B haplotypes are more diverse in the KIR gene content and are characterized by the presence of KIR2DL5, 2DL2, 2DS1, 2DS2, 2DS3, and/or 2DS5 genes [15]. KIR2DL4, 3DL2, 3DL3, and 3DP1 (so-called ‘framework’ genes) are present in both haplotypes and thus occur in all individuals [16].

Inter-individual variability in the KIR gene family depends on variation in gene number, particularly in a set of activating KIR genes and sequence polymorphism [17]. Variation in the genes and alleles may raise the question that genetic background influences susceptibility and resistance in autoimmune diseases. Consistent with this notion, specific KIR genotypes have been associated with a wide range of autoimmune diseases. The KIR2DS2/HLA-C1 genotype was reported to be associated with type I diabetes [18], the KIR2DS2*/KIR2DL2* [19] and KIR2DS1*/KIR2DS2* [20] genotypes were shown to be associated with susceptibility to systemic sclerosis, and KIR2DS1, KIR2DL5 receptors have been described as being associated with psoriasis susceptibility [21]. Additionally, genotypes with a greater number of activating KIR genes and a lower number of inhibitory KIR genes were reported to be associated with birdshot chorioretinopathy (BCR), Vogt-Koyanagi-Harada disease, and HLA-B27-associated acute anterior uveitis (AAU) and axial spondyloarthritis [22].

Hashimoto’s thyroiditis (HT) is the most common inflammatory disease of the thyroid gland as well as the most common autoimmune thyroid disease [23]. The incidence rate of HT has recently increased worldwide, with a higher prevalence reported in the elderly and lower in areas of iodine deficiency [24]. The etiology of HT is complex, with the interaction between internal (genetic) and external (environmental and endogenous) factors essential to disease initiation. HT is characterized by a gradual loss of thyroid function, the presence of a goiter and T cell infiltration observable on histological analysis. T cell infiltration into the thyroid gland and the interaction of T cells with thyroid antigens stimulate the secretion of inflammatory cytokines, resulting in tissue damage. Furthermore, studies have revealed the importance of mutation in the thyroglobulin and CTLA-4 genes in disease pathogenesis [25]. Likewise, the CTLA-4 polymorphism in the CTLA-4 3’UTR(AT)n repeat site has proven to be a key player in HT pathogenesis [26]. In addition, thyroid follicular cell damage induced by T cells results in autoantibodies against thyroid peroxidase and thyroglobulin secretion from B cells [27]. In the innate immune system, Solerte et al. reported a defect in NK cell cytokine secretions and cytotoxicity in HT patients. They suggest that the impairment of NK cell activity in HT disease could potentially determine a critical expansion of T/B cell immune compartments, leading to the generation of autoantibodies and to the pathogenesis of thyroid autoimmunity [28]. To examine the possibility that KIR genotypes contribute to the development of Hashimoto’s thyroiditis, we typed KIR genes in 118 patients and compared with the data of 120 healthy controls. This provided a system to assess the effect of NK cell receptors on autoimmune thyroiditis disease.

**Materials and methods**

**Study subjects**

A total of 118 patients with Hashimoto’s thyroiditis (mean age: 34.9 ± 12.6, 90% female and 10% male) and 120 healthy controls from the southern part of Iran (Fars province) were included in this study. The patients were recruited at Motahari polyclinic center, Shiraz University of Medical Sciences. However, HT was diagnosed on the basis of clinical symptoms and signs of hypothyroidism, depressed free T4/total T4 and elevated/normal TSH levels, diffuse goiter, and the presence of autoantibodies to thyroglobulin, microsomes (anti-TPO) or both [29, 30]. The HT clinical phenotype was confirmed based on the expertise of the referring HT specialist. The controls were age, sex, and ethnically matched to the patients. The study was reviewed and approved by the Medical Research Ethics Committee of Shiraz University of Medical Sciences. Genomic DNA was extracted from peripheral blood samples using a QIAamp blood kit (Qiagen, Hilden, Germany). The quality and quantity of DNA was determined by UV spectrophotometry, and the concentration was adjusted to 100 ng/μL.

**KIR genotyping**

The presence and absence of 11 KIR genes was determined by using a gene-specific polymerase chain reaction (PCR) typing system previously described [31]. Since the framework genes, KIR2DL4, 3DL2, 3DL3, and 3DP1, are invariably present in all individuals studied worldwide and KIR2DPI is present in most individuals, these KIR genes were excluded from this study. Unique genotypes were confirmed using a duplex SSP-PCR system [32, 33]. Furthermore, unusual genotypes were compared with worldwide genotype data in the “http://www.allelefrequencies.net” database [34]. Briefly, a different set of primers was used to type each KIR gene (primers provided by Dr. Rajalingam). PCR was performed in a reaction volume of 15 μl with a final concentration of 1× PCR buffer II (10 mM Tris–HCl and 50 mM KCl), 200 mM of each
The frequency of each genotype is expressed as a percentage and defined as the number of individuals having the genotype divided by the number of individuals studied. The frequencies of AA genotype and Bx (BB + AB) genotypes were predicted as described earlier [35]. Briefly, individuals carrying KIR2DL1-2DL3-3DL1-2DS4 present in the group A KIR haplotypes are considered homozygous for the A haplotypes and are designated as the AA genotype. Other individuals are regarded as carriers of Bx genotypes. Differences in distribution of KIR genes and genotypes were estimated by a two-tailed Fisher exact probability (p) test and p < 0.05 was considered to be statistically significant.

Results

The frequency of 11 variable KIR genes analyzed in 118 Hashimoto’s patients is illustrated in Table 1. There were no significant differences in the frequency of inhibitory and activating KIR genes between patients and healthy controls. We also compared the total number of inhibitory and activating KIR genes between HT patients and controls. Interestingly, we found more Hashimoto’s patients carrying six activating KIR (aKIR) genes compared to controls (11.8 % patient vs. 4.1 % in controls; $p = 0.032$, odds ratio (OR) = 3.09, 95 % confidence interval (CI) 1.07–8.89, Fig. 1). No statistical difference was observed in the number of inhibitory KIR genes and the rest of the activating KIR genes in patients versus controls.

### Table 1 Comparison of KIR gene frequencies and genotypes in patients with Hashimoto’s thyroiditis and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Healthy controls</th>
<th>Hashimoto’s thyroiditis patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 120</td>
<td>n = 118</td>
</tr>
<tr>
<td></td>
<td>%F (N)</td>
<td>%F (N)</td>
</tr>
<tr>
<td>AA</td>
<td>30.8 (37)</td>
<td>30.5 (36)</td>
</tr>
<tr>
<td>Bx</td>
<td>69.1 (83)</td>
<td>69.5 (82)</td>
</tr>
<tr>
<td>Inhibitory KIR genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2DL1</td>
<td>99.1 (119)</td>
<td>95.7 (113)</td>
</tr>
<tr>
<td>2DL2</td>
<td>60.0 (72)</td>
<td>55.1 (65)</td>
</tr>
<tr>
<td>2DL3</td>
<td>90.8 (109)</td>
<td>85.5 (101)</td>
</tr>
<tr>
<td>2DL5</td>
<td>59.1 (71)</td>
<td>55.9 (66)</td>
</tr>
<tr>
<td>3DL1</td>
<td>94.1 (113)</td>
<td>92.3 (109)</td>
</tr>
<tr>
<td>Activating KIR genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3DS1</td>
<td>33.3 (40)</td>
<td>35.6 (42)</td>
</tr>
<tr>
<td>2DS1</td>
<td>37.5 (45)</td>
<td>36.4 (43)</td>
</tr>
<tr>
<td>2DS2</td>
<td>55.8 (67)</td>
<td>56.7 (67)</td>
</tr>
<tr>
<td>2DS3</td>
<td>36.6 (44)</td>
<td>40.6 (48)</td>
</tr>
<tr>
<td>2DS4</td>
<td>94.1 (113)</td>
<td>93.2 (110)</td>
</tr>
<tr>
<td>2DS5</td>
<td>30.8 (37)</td>
<td>32.2 (38)</td>
</tr>
</tbody>
</table>

Frequency (%F) of each gene is expressed as a percentage and defined as the number of individuals having the gene (N) divided by the number of individuals studied (n) in the given population group.

The KIR gene content of a given individual is called the “KIR genotype”. We found 43 distinct KIR genotypes in the patients and controls studied. The overall difference in the distribution of KIR genotypes between controls and patients was not found to be statistically significant. Hashimoto’s patients exhibited 32 of 43 genotypes, 17 unique to the patient group (19.2 %), and three not previously reported in worldwide genotype data (genotypes 21, 28, and 31, Fig. 2) provided on the “http://www.allelefrequencies.net” database. Remarkably, the two genotypes (genotypes 4 and 6) carrying all six known activating KIR genes were found in increased frequency in the patient compared to the control population (genotypes 4: 7.6 vs. 3.3 %; genotypes 6: 4.2 vs. 0.8 %, Fig. 2).

No significant difference was seen in the frequency of AA genotype carriers and Bx genotypes carriers (AB and BB) between patients and control groups (Table 1).

Discussion

In the present study we described KIR gene frequency in thyroid autoimmune disease and demonstrated a genetic association between number of KIR genes and susceptibility to Hashimoto thyroiditis.
No significant association between inhibitory and activating KIR genes in patient and control were found that would indicate, at first glance, that the presence of KIR genes do not play a role in HT pathogenesis. We analyzed all combinations of KIR genes for the presence of more than one activating or inhibitory KIR genes. We hypothesize that the number of activating KIR genes may have an effect on susceptibility to HT. It appears that KIR activating function rather than specific activating KIR receptors predispose individuals to thyroid autoimmune disease. As expected from this hypothesis, the number of inhibitory KIR genes was not significantly different between patients and controls. NK cell function is regulated by a balance of activating and inhibitory signals. In some situations, the activating receptor signals are sufficient to stimulate NK cells regardless of inhibitory signal [3].

Consistent with our findings, several epidemiological studies have shown that the activating KIR receptors augment the susceptibility toward autoimmune response [36]. Furthermore, the association between activating KIR receptors and autoimmune eye diseases was reported in BCR and AAU [22]. Of particular interest, HaiQing et al. [37] showed that the KIR genotypes containing six inhibitory KIR genes occurred with less frequency in HT patients indicating less inhibition and perhaps more activating signals predispose individuals to Hashimoto thyroiditis susceptibility. Remarkably, Van der Silk et al. [38] reported no significant difference between KIR receptors in juvenile-onset type 1 diabetes patients and controls. In addition, they showed that activating KIR genotypes containing more than two genes influence susceptibility to type 1 diabetes [18]. Conversely, Augusto et al. [36] reported that higher levels of activating KIR signals appears to play a protective role in pemphigus foliaceus with high incidence in a specific region in Brazil but with low incidence worldwide, influenced by an environmental exposure which triggers the autoimmune disease. This finding is suggestive that carrying more activating KIR receptors may represent more susceptibility to some autoimmune diseases. Thus, an increase of NK cells with a greater number of activating receptors may augment the signal of NK activation for breaking a tolerance.

Shi et al. [39] showed that NK cell activation modifies antibody responses especially those against self-antigens, indicating a role for NK cells in B cell mediated autoimmunity. The infection etiology of several autoimmune diseases recruits the NK cells as a first line of defense to the site. Infection with Yersinia enterocolitica and hepatitis C virus reported to be related to development of HT [40]. NK cells with more activating KIR receptors may participate in the exposure of self-antigens through cell cytotoxicity and lysis. In addition, NK cells secrete cytokines such as IFN-γ that activate macrophages or skew the immune response toward a Th1 response. It is likely that triggering a NK cell response in first line of defense may lead to inflammation and autoimmune disease. Taken together, a greater number of activating genes may mean a larger repertoire of NK receptors recognizing antigens and influencing the NK cells to modulate the immune response.

Alternatively, it is known that activating KIR receptors are also expressed on a subset of CD8+ and CD4+ T cells. The KIR2DS2 receptor and DAP-12 activate cytotoxicity and cytokine production of T cells without TCR-derived signals [41]. In rheumatoid arthritis patients, the infiltration of the unusual TCD4+CD28− KIR+ cells to tissue lesions causes a rapid release of IFN-γ and result in cytotoxicity.
Additionally, the expression of stimulatory KIR receptors on T cells of Lupus patients causes an overproduction of IFN-\(\gamma\) [43]. These data clearly show that activating KIR receptors on T cells play a role in the pathogenesis of autoimmune diseases. Although found in only 12% of HT patients, increasing genotypes containing the six activating KIR receptors may increase T cell activation and contribute to HT pathogenesis.

The KIR gene frequency of our control group was similar to a previous study reported in the Persian population [35]. Interestingly, genotype 4 and 6 containing six activating KIR genes occur more frequently in Indian populations (Kanikar, Parsi, and Paravar) and Mexican populations [44–46], and although, two studies reported an increased prevalence of HT in the Indian and Mexican populations [47–49], the exact prevalence of the disease...
was not reported for the Indian subpopulations. Even though the Parsi population shares the same ancestry as the Persian population, with a high frequency of genotypes 4 and 6, no data are available to indicate the prevalence of HT in the Parsi. Thus, analyzing the prevalence of HT in the above populations with a high frequency of these two genotypes will bring more light to this discussion.

In summary, carrying six activating KIR genes appears to play a role in susceptibility to HT although a significant association was found in <12% of HT patients. As attractive as this data may be, the observed association must be validated by independent studies. Moreover, the role of inhibitory KIR and their relevant HLA class I ligands, as well as the functional implication of KIR-HLA combinations in the pathogenesis of Hashimoto’s thyroiditis, begs further study.

Acknowledgments This work was supported by a Grant (Grant No: 89-1-33-2015) from Shiraz University of Medical sciences, Shiraz, IRAN. We would like to thank Dr. Raja Rajalingam for providing primers and UCLA KIR Exchange DNA standards, Mrs. Sodah Rohanrad for technical assistance and Jeff M. Fortin and Linda L. Dunn for critical reading of the manuscript.

Conflict of interest The authors declare that there are no conflicts of interest.

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