Abstract
Objective: We aimed to determine the human leukocyte antigen (HLA) haplotypes of patients with autoimmune thyroiditis (AIT) and HLA group frequencies in clinical subgroups of disease and thus, investigate their role in the etiology of the disease.

Material and Method: One hundred patients with the diagnosis of AIT and 150 healthy controls with no known diseases were included in the study.

Results: HLA-DRB1*04 allele was significantly higher in the whole patient population, patients with hypothyroidism and/or atrophic thyroid gland compared to the control group, whereas there was no significant difference between patients with goitrous thyroiditis and clinically euthyroid compared to the control group. HLA-DQB1*0201 allele was significantly higher in the whole patient population and subgroups compared to the control group. HLA-DRB1*08 allele was defined as a protective locus due to its high positively rate in the control group compared to the whole patient population, and the presence of HLA-DRB1*12 allele was a protective factor, particularly for atrophic thyroiditis (AT). Evaluations of patients with hypothyroidism and AT showed that the development of hypothyroidism in clinical course of the disease was delayed in the presence of HLA-DQB1*02 allele.

Conclusion: The presence of AIT-RB1*04 and HLA-DQB1*02 alleles pose as risk factors for the development of AIT and especially of AT, whereas the presence of AIT-RB1*12 and HLA-DQB1*02 have protective roles, particularly in the development of AT, in our country.

Key words: Autoimmunity, Thyroiditis, Human leukocyte antigen

Introduction
Autoimmune thyroiditis (AIT) is one of the main causes of hypothyroidism and goiter in regions with sufficient dietary iodine intake (1). AIT has two clinical forms; called Hashimoto's thyroiditis (HT) when goiter is present or atrophic thyroiditis (AT) when the gland is atrophic. Both are characterized by the presence of auto-antibodies in serum and variable degrees of thyroid dysfunction (2). AIT develops as a result of the immune system’s response to the antigenic structures of thyroid. Immune response against the anti-
genetic structures of thyroid cells develops on the grounds of genetic factors with the contribution of environmental factors and results in tissue damage. Human Leukocyte Antigens (HLA) plays a major role in genetic contribution (3).

In AIT pathogenesis, HLA presents thyroid antigens to lymphocytes for initiation of immune response. Lymphocytes secrete cytokines and express intracellular adhesion and Fas molecules, which have important roles in intercellular interactions and apoptosis, respectively. The expression of HLA class II molecules on thyroid cells was first observed by Bottaza et al., who proposed viral infections in thyroid as initiation events for AIT that leads to stimulation of T lymphocytes and secretion of interferon-γ (IFN-γ) by the activated T lymphocytes, which in turn induce HLA class II expression (3). These events are currently believed to occur as a result of immune response rather than inducing immune response. Nevertheless, observations made by Bottaza and colleagues attracted attention onto the relationship between AIT and HLA class II molecules. HLA played important roles in the development of the disease in animal models. In mice, genes corresponding to human HLA loci were shown to be involved in the response of helper and suppressive T lymphocytes against certain antigens. HLA gene loci also regulate response of helper and suppressive T lymphocytes against certain antigens in humans and suppressive T lymphocytes for thyroid antigens were shown to be dysfunctional in individuals with certain genotypes. Hence, a genetically determined HLA-dependent antigen-specific suppressive T lymphocyte dysfunction was proposed as the main initiator of AIT in humans (4, 5).

HLA typing and nomenclature were modified within years and different phenotypes were defined in several studies. Genetic constitution of the populations under study was modified by migration, consanguinity and environmental factors. HLA studies are influenced by these factors (4).

Although studies on chronic AIT susceptibility determined by HLA class II genes were conducted in many populations, studies done in our country investigating the relationship between genetic factors and chronic AIT are scarce and small-scale (6, 7). In this study, we aimed to determine HLA class II haplotypes in chronic AIT patients and investigate an etiological role by studying the HLA group frequencies in clinical subgroups of disease.

Materials and Method

In this study, 100 patients with the diagnosis of chronic AIT based on clinical, laboratory, ultrasonographic and scintigraphic findings were included. As the control group, 150 healthy individuals (renal transplant donors) with no known diseases were included. The diagnosis of AIT was mainly ascertained by the detection of high levels of auto-antibodies in serum. The patient population was either hypothyroid or euthyroid based on the results of thyroid function tests done at the time of diagnosis. Ultrasonographic findings such as irregular appearance of thyroid gland decrease in gland size with concomitant increase in fibrosis or increase in gland size with widespread hypoechogenic appearance supported the diagnosis made by the presence of auto-antibodies. The heterogeneous distribution of radioactive material in thyroid scintigraphy and/or low uptake of radioactive material, irregular outline of thyroid gland, low uptake levels in patients undergoing radioactive iodine uptake test further supported the diagnosis.

Thyroid function tests

Thyroid hormone levels [free T3 (FT3), free T4 (FT4) and TSH] were measured by Abbott- Architect machine using chemiluminescence’s method. Serum Thyroglobulin (Tg), thyroid peroxidase anti-body (Anti-TPO Ab) and Thyroglobulin antibody (Anti-Tg Ab) levels were measured by commercial kits using radioimmunoassay method.

Ultrasonography of thyroid

Siemens Sonoline SL-1 with 7.5 MHz linear probe was used for ultrasonographic evaluation of thyroid. Width, depth and height of each lobe were measured. Thyroid volumes were also calculated. Volume for each lobe was calculated using the following formula (8);

\[ \text{Volume (mL)} = \frac{\pi}{6} \times \text{width} \times \text{height} \times \text{depth} \]

Total volume was determined by summation of lobe volumes.

Thyroid scintigraphy

Thyroid scintigraphy was taken using pinhole collimator gamma camera 20-40 minutes after intravenous injection of 5 mCi Tc 99m pertechnetate to the patients.

Method for tissue typing of HLA class II molecules

DNA isolation

Five mL blood from patients and healthy controls were collected in tubes with EDTA and centrifuged for 5 minutes at 1500 rpm. Genomic DNA from patient and control bloods was isolated using the denaturation-precipitation method with ammonium bromide salts. Isolated genomic DNAs were subjected to polymerase chain reaction (PCR) with commercially available primers (Olerup-SSP). Perkin-Elmer 9600 PCR machine was used. After PCR amplification, samples were separated on 2% agarose gel electrophoresis, stained with ethidium bromide and evaluated under UV light. DNA samples were typed as HLA-DRB1 and DQB1 after evaluation.

Statistics

SPSS Windows 10.0 package was used for statistical tests in the study. Student t test for independent samples was used to compare group averages. Data were expressed as mean ± standard error. p values with less than 0.05 were considered significant. Chi-square test or if needed, Fisher’s exact chi-square test, were used to test the significance of differences in ratios between patient and control groups having certain tissue phenotypes. Odds ratio (OR) and 95% confidence interval for OR were calculated to determine the risk posed by having certain HLA tissue types.

Results

Ninety-one percent (91%) of patients were women and 9% were men with 43 ± 1.2 (range = 16-75) years of age at the time of diagnosis. Thirty-nine percent (39%) of the cases were euthyroid, while 61% were hypothyroid at the time of diagnosis. Fifty-one percent (51%) of the cases were of males, while 49% were non-goitrous thyroiditis. The comparative data of patients based on thyroid function tests and thyroid gland size are shown in Table 1 and 2.

HLA-DRB1*04 allele in HLA-DR tissue group was detected with highest frequency (35%) in patients with AIT. This allele was the most common allele present in controls. The strength of association between patients and controls with regard to HLA-DRB1*04 allele positivity (p = 0.034). According to the odds ratio, the probability of contracting the disease by individuals with this allele was 1.837.
times higher than by the individuals without this allele. HLA-DRB1*08 allele was detected in 3% of the patients while 12% of the controls were positive for this allele; this difference was statistically significant (p = 0.02). DQB1*03 allele in HLA-DQ tissue group was the most frequent group with a rate of 53% in AIT patients. This group was positive in 50.6% of the controls and the difference between patient and control group was not statistically significant (p = 0.718). While DQB1*0201 allele was detectable in 6% of the patients, control group was negative for this allele. The difference between patient and control groups was statistically significant (p = 0.009).

When HLA-DR tissue group was examined in the clinically euthyroid patient population, the most frequent allele was again HLA-DRB1*04 with 33.3%. However, when patient and control groups were compared for HLA-DRB1*04 positivity, the difference was not statistically significant (p = 0.173). When HLA-DQB1 tissue group positivity was examined, DQB1*0201 allele was determined at a frequency of 5.1% in the patient population. The difference between groups in terms of DQB1*0201 positivity was statistically significant (p = 0.042).

When HLA-DR tissue group was examined in patients with clinical hypothyroidism, HLA-DRB1*04 allele was the most frequent with 36.1% positivity. The difference between patient and control groups was statistically significant for this allele (p = 0.047). HLA-DRB1*08 allele was detected in 3.3% of patients with hypothyroidism. The difference between patient and control groups was not statistically significant (p = 0.068). HLA-DQB1*02 allele among HLA-DQ tissue group was detected in 13.1% and 26.6% of patient and control groups, respectively. The difference between groups in terms of HLA-DQB1*02 positivity was statistically significant (p = 0.037). The most significant finding for correlation between HLA and hypothyroid AIT was the positivity of HLA-DQB1*0201 allele in 6.5% of the patients while controls were completely negative (p = 0.007).

There was no statistical difference between controls and patients with goitrous AIT for allelic frequencies of HLA-DR and DQ. However, when groups were compared for HLA-DRB1*08 and HLA-DQB1*02 alleles, which were previously found to be probable protective factors, they were not significant statistically (p = 0.065 and p = 0.063, respectively).

When HLA-DQ tissue group was examined between AIT patients with normal sized or atrophic thyroid glands and controls, HLA-DRB1*04 was the most frequent allele in the patient population with a rate of 42.8%. The difference was statistically significant when compared to the control group (p = 0.007), while OR was calculated as 2.559.

Another interesting finding in this group was the complete absence of DRB1*12 allele of HLA-DR group in the patient population, while 11.3% of the controls were positive for this allele. The difference between groups was statistically significant (p = 0.008). When HLA-DQ antigens were examined, HLA-DQB1*03 allele was found positive in 49% of the patients. However, 50.6% of the controls were also positive for this allele and the difference was not statistically significant (p = 0.838). On the other hand, DQB1*0201 allele was positive in 8.1% of the patients while none of the controls were positive for this allele. The differences between groups were highly significant (p = 0.003).

A general evaluation of the results showed that a correlation exists between HLA histocompatibility antigens and AIT, particularly of those with hypothyroidism or atrophy in clinical course. For this reason, we generated a new group consisting of patients with clinical hypothyroidism and with less-than-normal sized thyroid glands (i.e. primary myxedema, atrophic thyroiditis). In this group, there were 41 patients with 3 men and 38 women. The age at the time of diagnosis was 47.58 ± 19 years (range = 18-75 years). The average thyroid gland volume of the patients was 9.53 ± 0.68 mL. TSH, anti-TPO Ab and anti-Tg Ab were determined as 47.25 ± 12.72 mIU/mL, 2219.63 ± 402.62 U/mL and 839.01 ± 246.43 IU/mL (mean ± standard error), respectively. The allelic distribution fre-

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Table 1. Comparative data of patient population based on thyroid functions

<table>
<thead>
<tr>
<th>Euthyroid</th>
<th>Hypothyroid</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>39 (39.0)</td>
<td>61 (61.0)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.05 ± 1.67</td>
<td>46.11 ± 1.66</td>
</tr>
<tr>
<td>TSH (mIU/ml)</td>
<td>1.63 ± 0.20</td>
<td>41.17 ± 9.14</td>
</tr>
<tr>
<td>Anti-TPO Ab (IU/ml)</td>
<td>2016.63 ± 369.76</td>
<td>2159.80 ± 324.69</td>
</tr>
<tr>
<td>Anti-Tg Ab (IU/ml)</td>
<td>741.82 ± 257.47</td>
<td>784.15 ± 183.64</td>
</tr>
<tr>
<td>Thyroid volume</td>
<td>20.48 ± 1.80</td>
<td>13.54 ± 1.26</td>
</tr>
</tbody>
</table>

Table 2. Comparative data of patient population based on thyroid gland size

<table>
<thead>
<tr>
<th>Goitrous</th>
<th>Non-goitrous</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>51 (51.0)</td>
<td>49 (49.0)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.07 ± 1.69</td>
<td>45.97 ± 1.80</td>
</tr>
<tr>
<td>TSH (mIU/ml)</td>
<td>12.15 ± 4.18</td>
<td>39.9 ± 10.89</td>
</tr>
<tr>
<td>Anti-TPO Ab (IU/ml)</td>
<td>2243.71 ± 344.31</td>
<td>1958.51 ± 347.86</td>
</tr>
<tr>
<td>Anti-Tg Ab (IU/ml)</td>
<td>779.84 ± 215.95</td>
<td>734.13 ± 208.96</td>
</tr>
<tr>
<td>Thyroid volume</td>
<td>22.59 ± 1.63</td>
<td>9.64 ± 0.58</td>
</tr>
</tbody>
</table>
quences of tissue histocompatibility antigens were compared with the control group. HLA-DRB1*04 was the most frequent tissue group allele in the patient population with a positivity rate of 41.4%. The difference was statistically significant when compared with the control group \( (p = 0.018) \). OR was calculated as 2.417.

When the whole patient population was evaluated, HLA-DRB1*08 allele emerged as a protective factor \( (p = 0.02) \). While this allele was positive in 4.8% of patients with atrophic thyroiditis, control group had 12% positivity rate, but this difference was not statistically significant \( (p = 0.202) \).

As seen in AIT patients with normal sized or atrophic thyroid glands, HLA-DRB1*12 allele was also negative in patients with atrophic thyroid glands and hypothyroidism, while the positivity rate of this allele in the control group was 11.3%. This difference between groups was statistically significant \( (p = 0.013) \). While there was no difference for HLA-DQB1*02 frequency between AIT patients with normal sized or atrophic thyroid glands and the control group, it was important not to find this allele in patients with atrophic thyroiditis in spite of the fact that this allele was known as another protective factor in patients with hypothyroidism. The difference between patient and control groups was also significant \( (p = 0.03) \). While 7.3% of the patient population was positive for DQB1*0201 allele of HLA-DQ1, the control group was negative. The difference between groups was statistically significant \( (p = 0.009) \).

### Discussion

Although there are no strong epidemiological indications for the contribution of genetic factors to AIT etiopathogenesis, a few hereditary factors have been described by investigating clues at hand. The most widely studied factors on this subject are tissue histocompatibility antigens (4). We have investigated the haplotypes of HLA class II in patients with chronic AIT diagnosis. The frequency of HLA-DRB1*04 allele was significantly higher in the whole patient population and patients with hypothyroidism or atrophic thyroid glands compared to the controls. There was not a significant difference between clinically euthyroid patients with goitrous thyroiditis and the control group. Accordingly, HLA-DRB1*04 allele could be proposed as an effective locus in the etiology of AIT in Turkey.

Moreover, HLA-DQB1*0201 allele among HLA-DQB1 tissue antigen haplotypes was found significantly higher in the subgroups of disease (euthyroid, hypothyroid and atrophic thyroiditis patients) compared to the control group. Our data supports the notion that in the presence of this allele the development of AIT, particularly of those progressing to hypothyroidism or atrophy is facilitated.

When the whole patient population was evaluated, HLA-DRB1*08 allele was defined as a protective locus due to its high positivity rate in the control group. Although the evaluations in subgroups suggested that in the presence of this allele the development of AIT, particularly of those with goitrous or hypothyroid course was less likely; however, this notion was not supported statistically. Our study showed that another protective factor from AIT was the presence of HLA-DRB1*12 allele. Hence, the negativity of HLA-DRB1*12 allele might be rendering AIT development more likely in our country. Based on evaluations made in subgroups (patient populations with hypothyroidism and AIT), the presence of HLA-DQB1*02 allele was determined as a restrictive factor for development of hypothyroidism in the course of disease (Table 3).

### Table 3: HLA Class II markers and risk or protective factors for AIT

<table>
<thead>
<tr>
<th></th>
<th>Risk factors</th>
<th>Protective factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLA-DR</td>
<td>HLA-DQ</td>
</tr>
<tr>
<td>All patients</td>
<td>DRB1*04</td>
<td>DQB1*0201</td>
</tr>
<tr>
<td>Euthyroid</td>
<td>None</td>
<td>DQB1*0201</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>DRB1*04</td>
<td>DQB1*0201</td>
</tr>
<tr>
<td>Goitrous</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Non-goitrous</td>
<td>DRB1*04</td>
<td>DQB1*0201</td>
</tr>
<tr>
<td>Atrophic AIT</td>
<td>DRB1*04</td>
<td>DQB1*0201</td>
</tr>
</tbody>
</table>

Studies with more comprehensive HLA typing revealed HLA-DQ7 allele with an OR of 4.7 as the strongest correlate (13). HLA-DR4 was reported to correlate with HT in other studies conducted in white race (14-17). In a meta-analysis based on HLA series data from English population, OR was found low (-2.3 and 1.6 for HLA-DR3 and HLA-DR4, respectively), and no correlation was evident between DR/DQ alleles and disease (18). In a study conducted on Japanese population, a correlation was detected between the disease and HLA-B46/HLA-DR9 alleles (19). In another study conducted on Japanese population, HLA-DRw53 allele was also found to correlate with HT (20). HLA-Bw46 was detected frequently in Chinese patients from Shanghai having goitrous HT (21). In another study conducted on southern Chinese, a correlation was detected between HT and...
and geography. We believe that the studies investigating correlations between settlements in different populations grounds, alleles might be effecting differentially due to environmental factors for disease development.

We did not detect any specific locus that increased or decreased as a protective factor for AIT, while HLA-DRB1*12 and HLA-DQB1*02 alleles.

Our study indicated that the positivity HLA-DRB1*04 and HLA-DQB1*03 alleles.

Not only our study is the most comprehensive one ever conducted on Turkish population, but also the first one for using DNA analysis techniques. Our results support the correlation between AIT and HLA-DR4 that were detected by Oz bey et al. using serologic techniques.

We studied that the positivity HLA-DRB1*04 and HLA-DQB1*02 alleles pose as risk factors for the development of AIT, especially AT. Moreover, the positivity of HLA-DRB1*08 allele acts as a protective factor for AIT, while HLA-DRB1*12 and HLA-DQB1*02 alleles have protective roles from AT development in particular. We did not detect any specific locus that increased or decreased the susceptibility risk for contracting goitrous AIT. Considering the existence of an endemic goiter region in our country, the iodine prophylaxis may also have contributed to the disease development process, thus these cases may have been influenced by the environmental factors for disease development.

HLA system constitutes only a small fraction of genetic factors in AIT etiology. Besides, even in groups with same ethnic backgrounds, alleles might be effecting differentially due to environmental factors stemming from settlements in different populations and geography. We believe that the studies investigating correlations between AIT and HLA should continue in parallel to the advances made in molecular biology and in the light of new discoveries regarding human genome.

References