Association of the polymorphisms in Th2 chemotaxis-related genes with the development and prognosis of autoimmune thyroid diseases

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Abstract. The prognosis of autoimmune thyroid disease (AITD) is difficult to predict. Th2 cells suppress the differentiation of Th1 and Th17 cells, which are associated with the prognosis of AITD. However, there are few reports as to whether Th2 chemotaxis-related genes, such as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), IL-25, TARC/CCL17 (Thymus and activation regulated chemokine/chemokine ligand 17) or STAT6 (Signal transducer and activator of transcription 6), affect the pathology of and/or susceptibility to AITD. Therefore, in this study, we genotyped functional SNPs in these genes to clarify the association of the genetic differences of genes related to Th2 differentiation and chemotaxis with the development and the prognosis of AITDs. The frequencies of the AA genotype of the CRTH2 rs545659 SNP and the CC genotype and the C allele of the CRTH2 rs634681 SNP were higher in patients with severe HD than in patients with mild HD. The frequency of the CC genotype in the TARC rs223828 SNP was higher in patients with intractable GD than in patients with GD in remission. In conclusion, the CRTH2 rs545659 and rs634681 SNPs were associated with the severity of HD, and the TARC/CCL17 rs223828 SNP was associated with the intractability of GD.

Key words: Th2, autoimmune thyroid disease, severity, intractability, polymorphism

AUTOIMMUNE THYROID DISEASES (AITDs), such as Graves’ disease (GD) and Hashimoto’s disease (HD), are archetypal organ-specific autoimmune diseases [1, 2]. The intractability of GD and the severity of HD varies among patients and are very difficult to predict at diagnosis. CD4⁺ helper T (Th) cells have been classified into at least three different subsets, Th1, Th2 and Th17, based on their patterns of cytokine production [3-7]. We have already reported that genetically higher expressions of Th1 cytokines and/or lower expressions of Th2 cytokines, and high Th1/Th2 ratio were associated with the severity of HD [8-10]. On the other hand, genetically higher expressions of Th17 cytokines and high proportion of Th17 cells were associated with intractability of GD [10-12].

Th2 cells produce specific cytokines, such as Interleukin (IL)-4, IL-5, and IL-13, and are known to be associated with immunity [13-15]. Th2 cells may suppress the activation of Th1 cytokines and/or lower expressions of Th2 cytokines, and high Th1/Th2 ratio were associated with the severity of HD [8-10]. On the other hand, genetically higher expressions of Th17 cytokines and high proportion of Th17 cells were associated with intractability of GD [10-12].

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Abbreviation: AITDs, autoimmune thyroid diseases; GD, Graves’ disease; HD, Hashimoto’s disease; CD, cluster of differentiation; Th, helper T; IL, interleukin; CRTH2, chemoattractant-receptor homologous molecule expressed on Th2 cells; TARC, thymus and activation regulated chemokine; STAT6, Signal transducer and activator of transcription 6; SNPs, single nucleotide polymorphisms; AML-1, acute myeloid leukemia-1; CRE-BP, Cyclic-AMP response element binding protein; C/EBPα, CCAAT/enhancer binding protein alpha; TRAB, anti-thyrotrophin receptor antibody; TgAb, anti-thyroglobulin antibody; McAb, anti-thyroid microsomal antibody; TPOAb, anti-thyroid peroxidase antibody; EDTA, ethylenediamine tetraacetic acid; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; PBS, phosphate buffered saline; MACS, magnetic-activated cell sorting

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severity of HD and/or the intractability of GD because Th2 cytokines suppress the differentiation and the activation of Th1 and Th17 cells [16-18]. Major genes expressed in Th2 cells, such as CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), IL-25, TARC/CCL17 (Thymus and activation regulated chemokine/chemokine ligand 17) and STAT6 (Signal transducer and activator of transcription 6), are naturally important factors for Th2 immune responses [19-25].

CRTH2 is a G-protein-coupled receptor for prostaglandin D2 (PGD2) [26]. PGD2-CRTH2 signaling induces chemotaxis and expression of Th2 cytokines and is important for allergic inflammation such as asthma [27-29]. The single nucleotide polymorphisms (SNPs) in the CRTH2 gene, rs533116, rs545659, rs634681 and rs11571288, have been associated with asthma and allergic sensitization and the functions of these polymorphisms were shown in Table 1 [30-35]. Therefore, these CRTH2 SNPs may be associated with chemotaxis of Th2 cells to thyroid gland and induction of Th2 differentiation through IL-4 and IL-13 signaling. IL-25 (also known as IL-17E) is a member of the IL-17 cytokine family, but IL-25 is identified as a proinflammatory cytokine that promotes Th2 responses by inducing Th2 cytokines [36, 37]. Although the function of the rs7145531 SNP in the IL25 gene is still unknown, this polymorphism may be associated with transcription activity and gene expression because the C allele provides a binding site for acute myeloid leukemia-1 (AML-1) and Cyclic-AMP response element binding protein (CRE-BP), whereas the T allele provides a binding site for CCAAT/enhancer binding protein α (C/EBPα) [38]. TARC/CCL17 induces the recruitment and migration of Th2 cells, affecting the susceptibility of Th2-associated disease such as atopic dermatitis and bronchial asthma [39, 40]. The frequency of the T allele of the rs223828 SNP, which has higher transcription activity, was lower in patients of Kawasaki disease and multiple sclerosis than in controls [41-43]. Low frequency of T allele may promote Th1 reaction which was associated with pathogenesis of multiple sclerosis [43]. On the other hand, although T allele was less frequent in patients with Kawasaki disease, TARC level was higher in them than in control subjects [42], but this may be because duration of fever in these patients were longer than in controls [42]. Therefore, we suggest that this SNP may be associated with chemotaxis of Th2 cells. Since IL-4 secreted by Th2 cells may promote TARC expression via STAT6 signaling [44], migration of Th2 cells to thyroid gland may be enhanced. STAT6 is an important molecule in the IL-4/IL-4 receptor signal transduction pathway associated with Th2 cytokine production [45]. STAT6 is one of the transcription factors that also binds to the promotor region in the TARC/CCL17 gene [44]. We genotyped rs324011 and rs324015 SNPs in the STAT6 gene, the functions of these polymorphisms were shown in Table 1 [45-48].

There are few reports of whether these SNPs also affect the pathology of and/or susceptibility toAITD. In this study, therefore, we genotyped these functional SNP genes to clarify the association of the genetic differences of these genes important for Th2 differentiation and chemotaxis with the development and prognosis ofAITDs.

### Materials and Methods

#### Subjects for genotyping

We screened each polymorphism in 421 AITD patients and 141 healthy volunteers. Among 214 GD patients who had a clinical history of thyrotoxicosis and were positive for anti-thyrotophin receptor antibody (TRAb),
we screened 108 patients who had been treated with methimazole for at least 5 years and were still positive for TRAb (intractable GD), 55 patients with GD who had maintained a euthyroid state and were negative for TRAb for more than 2 years without medication (GD in remission), and 51 patients who could not be categorized to either the intractable GD or GD in remission group at the time of analysis.

We categorized HD patients expeditiously using their ages to develop hypothyroidism and compared patients’ groups with typically different prognosis. Among 207 HD patients who were positive for anti-thyroglobulin antibody (TgAb) and/or (anti-thyroid microsomal antibody (McAb) or anti-thyroid peroxidase antibody (TPOAb)), we genotyped 98 patients who developed moderate to severe hypothyroidism before 50 years of age and were treated with thyroxine (The rate of thyroid destruction is fast; severe HD), 51 untreated euthyroid patients who were over 50 years of age (The rate of thyroid destruction is slow; mild HD) and 58 patients who could not be categorized to either the severe HD or mild HD group at the time of analysis. Healthy volunteers were euthyroid and negative for any thyroid autoantibodies. Genomic DNA was isolated from ethylenediaminetetraacetic acid (EDTA)-treated whole blood cells with a commercially available kit (QIAamp® DNA Blood Mini Kit, QIAGEN, Tokyo, Japan). Written informed consent was obtained from all the patients and control subjects, and the study protocol was approved by the Ethics Committee of Osaka University (564). Clinical characteristics were shown in Table 2.

### Genotyping of polymorphisms

Genotyped SNPs in this study are shown in Table 1. We used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for genotyping the CRTH2 rs533116 C/T, rs545659 A/G, rs634681 C/T, IL25 rs7145531 C/T, TARC rs223828 C/T, and STAT6 rs324011 C/T SNPs. The target sequences of each gene were amplified using PCR, and the PCR product was digested by the addition of each restriction enzyme. The sequences of forward and reverse primers, the PCR conditions and restriction enzyme used in this study are summarized in Table 3. We also used TaqMan®

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**Table 2** Clinical characteristics of the subjects at the sampling

|                     | Controls | GD (Past clinical history of thyrotoxicosis with elevated TRAb) | HD (Diffuse goiter and positive TgAb and/or McAb) |
|---------------------|----------|---------------------------------------------------------------|---------------------------------------------------|
|                     |          | intractable | remission | severe | mild |
| n (female/male)     | 141 (102/39) | 108 (88/20) | 55 (49/6) | 98 (80/18) | 51 (43/8) |
| The age of onset (years) | 35.0 ± 14.2 | 33.8 ± 13.8 | 33.6 ± 14.8 | 38.7 ± 10.5 | 62.2 ± 10.0 |
| Free T4 (ng/dL)      | ND       | 1.2 ± 0.3 | 1.2 ± 0.2 | 1.3 ± 0.3 | 1.2 ± 0.2 |
| Free T3 (pg/mL)      | ND       | 2.8 ± 0.7 | 2.6 ± 0.3 | 2.6 ± 0.5 | 2.8 ± 0.4 |
| TSH (μU/mL)          | ND       | 1.5 ± 1.3 | 1.8 ± 1.2 | 3.7 ± 9.8 | 2.7 ± 1.7 |
| TRAb (IU/L)          | <2.0     | 13.6 ± 58.5 | 13.6 ± 58.5 | 13.6 ± 58.5 | 13.6 ± 58.5 |
| TgAb (2^8 × 100)^-a | Negative | 2.8 ± 3.0 | 2.9 ± 3.2 | 6.6 ± 3.2 | 1.3 ± 2.4 |
| McAb (2^8 × 100)^-a  | Negative | 4.4 ± 2.7 | 5.4 ± 2.1 | 5.4 ± 3.0 | 3.5 ± 2.8 |
| Current treatment    | None     | MMI or PTU | None | L-thyroxine | None |
| Duration of treatment (years) | None | 11.2 ± 7.7 | 3.1 ± 1.3^-d | 8.7 ± 8.8 | None |
| Current dose of anti-thyroid drug (mg/day)^-b | None | 16.4 ± 28.7 | None | None | None |
| Current dose of L-thyroxine drug (μg/day) | None | None | None | 80.2 ± 37.2 | None |

Data are expressed as mean ± standard deviation. ND, not determined; PTU, propylthiouracil; MMI, methimazole
^-a When the titer of TgAb or McAb was 25,600, it was expressed as 2^8 × 100. ^-b Doses were expressed as the comparable dose of MMI (50 mg of PTU was converted to 5 mg of MMI). ^-c Age at the time of sampling. ^-d Duration of the treatment with antithyroid drug before remission
SNP Genotyping Assay (Applied Biosystems, Tokyo, Japan) to genotype the CRTH2 rs11571288 C/G and STAT6 rs324015 C/T SNPs.

mRNA extraction
We evaluated expression levels of STAT6 mRNA in 10 patients with GD in remission, 10 patients with mild HD and 15 control subjects to exclude the effect of medication. Peripheral blood mononuclear cells (PBMCs) were isolated from heparin-treated peripheral blood. In brief, peripheral blood was isolated by density gradient centrifugation with Lymphoprep (density 1.077, Nycomed Phama As, Oslo, Norway) at 400 g for 30 minutes at room temperature and washed in phosphate buffered saline (PBS). We separated CD4+ cells from PBMCs by magnetic-activated cell sorting (MACS). CD4+ cells were resuspended at 5 × 10^5–1 × 10^6/mL in Roswell Park Memorial Institute medium (RPMI) containing 10% fetal bovine serum and were cultured for 2 hours at 37°C in a humidified atmosphere containing 5% CO2, with 10 μg/mL of brefeldin-A (Sigma, St Louis, MO, USA) plus 25 ng/mL of phorbol myristate acetate (Sigma), and 1 μg/mL of ionomycin (Sigma). We extracted mRNA from CD4+ cells using a commercially available kit (RNAiso, Takara Bio Inc., Shiga, Japan).

Quantitative RT-PCR
STAT6 cDNAs were generated using High Capacity cDNA Reverse Transcription Kit (Life Technologies, Tokyo, Japan). Real-time PCR was performed with Taqman® Gene Expression Assays (Life Technologies, Tokyo, Japan). Expression levels of STAT6 mRNA were normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All reactions were performed in triplicate. The relative expression levels of each mRNA were calculated using the ∆∆Ct method and shown as the relative value to a particular sample.

Statistical analysis
We used a χ2 test and Fisher’s exact test to evaluate the significance of differences in the frequencies of genotypes and alleles among the groups. A Kruskal-Wallis test was used to analyze the differences between the titers of MeAb and TgAb and the levels of TRAb among each genotype. Student’s t-test was used to analyze the difference in STAT6 expressions in CD4+ cells. Data were analyzed with JMP12 software (SAS Institute Inc., Tokyo, Japan). Probability values of <0.05 were considered significant.

### Table 3
| Primer pairs | PCR conditions | Restriction enzymes |
|--------------|----------------|---------------------|
| rs533116     | 95°C for 5 min | BsoBI               |
|              | (95°C for 30 sec, 63°C for 30 sec, 72°C for 30 sec) × 30 cycles |                      |
|              | 72°C for 5 min |                     |
| rs546569     | 95°C for 5 min | NlaIII              |
|              | (95°C for 30 sec, 54.8°C for 30 sec, 72°C for 30 sec) × 35 cycles |                      |
|              | 72°C for 5 min |                     |
| rs634681     | 95°C for 5 min | EcoRI               |
|              | (95°C for 30 sec, 63.5°C for 30 sec, 72°C for 30 sec) × 33 cycles |                      |
|              | 72°C for 5 min |                     |
| rs11571288   | Taqman® SNP Genotyping Assays (Applied Biosystems, Tokyo, Japan) |                      |
| rs7145531    | 95°C for 5 min | MnlI                |
|              | (95°C for 30 sec, 56.6°C for 30 sec, 72°C for 30 sec) × 35 cycles |                      |
|              | 72°C for 5 min |                     |
| rs223828     | 95°C for 5 min | Sall                |
|              | (95°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec) × 33 cycles |                      |
|              | 72°C for 5 min |                     |
| rs324011     | 95°C for 5 min | AvaII               |
|              | (95°C for 30 sec, 60.2°C for 30 sec, 72°C for 30 sec) × 30 cycles |                      |
|              | 72°C for 5 min |                     |
| rs324015     | Taqman® SNP Genotyping Assays (Applied Biosystems, Tokyo, Japan) |                      |
Results

**CRTH2 rs545659 A/G SNP**

The frequency of the GG genotype of the *CRTH2* rs545659 SNP was lower in patients with GD than in control subjects \((p = 0.0247)\) (Table 4). However, we found no significant difference in the frequency of allele between GD and control subjects. Moreover, the frequency of the AA genotype was higher in patients with severe HD than in mild HD \((p = 0.044)\) (Table 5). We did not also find any differences in the frequencies of genotype or allele between patients with HD and normal subjects or between intractable GD and GD in remission.

**CRTH2 rs634681 C/T SNP**

The frequencies of the CC genotype and the C allele of the *CRTH2* rs634681 SNP were higher in patients with severe HD than in patients with mild HD \((p = 0.0044\) and 0.042, respectively) (Table 5). We did not find any differences in genotype or allele frequencies between GD patients and control subjects or between HD patients and control subjects (Table 4).

**CRTH2 rs533116 C/T and rs11571288 C/G SNPs**

We did not find any differences in genotype or allele frequencies of either the *CRTH2* rs533116 or rs11571288 SNP between control subjects and each patient’s group of GD and HD. The frequencies of genotypes and alleles in these SNPs also did not differ between the patients with intractable GD and those with GD in remission or between the patients with severe HD and those with mild HD (Tables 4, 5).

**IL25 rs7145531 C/T SNP**

We did not find any differences in genotype or allele frequencies of the *IL25* rs7145531 SNP between control subjects and each patient’s group of GD and HD. The frequencies of genotypes and alleles in this SNP also did not differ between the patients with intractable GD and those with GD in remission or between the patients with severe HD and those with mild HD (Tables 4, 5).

**TARC rs223828 C/T SNP**

The frequency of the CC genotype in the *TARC* rs223828 SNP was higher in patients with intractable GD than in patients with GD in remission \((p = 0.0196)\) (Table 7). However, we did not find significant difference in allele frequency between intractable GD and GD in remission. We did not find any differences in genotype or allele frequencies between GD patients and control subjects or between HD patients and control subjects (Table 6).

**STAT6 rs324011 C/T and rs324015 C/T SNPs**

We did not find any differences in genotype or allele frequencies of the *STAT6* rs324011 or rs324015 SNP between control subjects and each patient’s group with GD and HD. The frequencies of genotypes and alleles in these SNPs also did not differ between patients with intractable GD and those with HD in remission or between the patients with severe HD and those with mild HD (Tables 6, 7).

The expression levels of *STAT6* mRNA in CD4+ cells

The expression levels of *STAT6* mRNA did not show any significant differences among each genotype of the *STAT6* rs324011 or rs324015 SNP (data not shown).

Discussion

In this study, we expected that the polymorphisms in Th2-related genes could be associated with the susceptibility and the prognosis of AITDs because we had already found that the Th1/Th2 ratio was associated with the severity of HD [12], and the proportion of Th17 cells, which are suppressed by Th2 cytokines, was associated with the susceptibility of AITD and the intractability of GD [12]. Expectedly, the *CRTH2* rs545659 polymorphism was associated with the susceptibility of GD, the *CRTH2* rs545659 and rs634681 polymorphisms were associated with the HD severity, and the *TARC/CCL17* rs223828 polymorphism was also associated with the intractability of GD (Tables 4, 5, 7).

In the case of the *CRTH2* gene, we expected that the frequency of the AA genotype of the rs545659 SNP would be higher in patients with severe HD and intractable GD than those with mild HD and GD in remission, respectively, as the A allele of this SNP may show lower expression level of CRTH2 and lower proportion of Th2 cells than the G allele [32, 34]. Expectedly, the frequency of the AA genotype was higher in patients with severe HD than in mild HD (Table 5). The frequency of AA genotype in severe HD (11.94%) was similar in controls (10.23%) and was not associated with the development of HD (Table 5). Therefore, we hypothesize that CRTH2 may not contribute to the breakdown of thyroid-specific immunological tolerance but that once autoimmune destruction of the thyroid gland starts, low CRTH2 expres-
sion may accelerate the destruction of thyroid gland by activating cytotoxic T cells.

Contrary to expectation, the frequency of A carrier (GA + AA genotypes), which shows lower expression of the CRTH2 gene, was higher in patients with GD (Table 4), and there was no association of this SNP with the intractability of GD. This may be because in patients with GD, who show a higher proportion of Th17 cells [12], the expression level of CRTH2, which induces Th2 cytokine expression [30], and the suppression of Th17...
cells by Th2 cells may be too weak to affect the GD intractability [16, 18]. In the case of the rs634681 SNP, the frequencies of the T allele and the T allele carrier were higher in patients with mild HD than those with severe HD (Table 5). These results suggest that HD patients with the T allele show a higher proportion of Th2 cells because of higher expression level of the CRTH2 gene [35]; therefore, these patients may show

Table 5  Genotype and allele frequencies of CRTH2 polymorphisms genotyped in this study in patients with HD, GD

| CRTH2 rs533116 | control | GD | HD |
|---------------|---------|----|----|
|               |         |    |    |
| CC            | 90 (92.78%) | 76 (87.36%) | 41 (87.23%) |
| CT            | 7 (7.22%) | 11 (12.64%) | 6 (12.77%) |
| TT            | 0 (0%) | 0 (0%) | 0 (0%) |
| CC + CT       | 90 (92.78%) | 76 (87.36%) | 41 (87.23%) |
| TT            | 0 (0%) | 0 (0%) | 0 (0%) |
| C allele      | 187 (96.39%) | 163 (93.68%) | 88 (93.62%) |
| T allele      | 7 (3.61%) | 11 (6.32%) | 6 (6.38%) |

| CRTH2 rs545659 | control | GD | HD |
|---------------|---------|----|----|
|               |         |    |    |
| GG            | 46 (52.27%) | 25 (34.72%) | 17 (37.78%) |
| GA            | 33 (37.5%) | 43 (59.72%) | 25 (55.55%) |
| AA            | 9 (10.23%) | 4 (5.56%) | 3 (6.67%) |
| GG + GA       | 46 (52.27%) | 25 (34.72%) | 17 (37.78%) |
| AA            | 9 (10.23%) | 4 (5.56%) | 3 (6.67%) |
| G allele      | 125 (71.02%) | 93 (64.58%) | 59 (65.56%) |
| A allele      | 51 (28.98%) | 51 (35.42%) | 31 (34.44%) |

| CRTH2 rs634681 | control | GD | HD |
|---------------|---------|----|----|
|               |         |    |    |
| CC            | 9 (9.38%) | 15 (17.24%) | 5 (10.64%) |
| CT            | 36 (37.5%) | 29 (33.33%) | 19 (40.42%) |
| TT            | 51 (53.12%) | 43 (49.43%) | 23 (48.94%) |
| CC + CT       | 9 (9.38%) | 15 (17.24%) | 5 (10.64%) |
| TT            | 87 (90.62%) | 72 (82.76%) | 42 (89.32%) |
| C allele      | 138 (71.87%) | 115 (66.09%) | 65 (69.15%) |
| T allele      | 51 (28.13%) | 51 (35.42%) | 31 (34.44%) |

| CRTH2 rs11571288 | control | GD | HD |
|-----------------|---------|----|----|
|                 |         |    |    |
| GG              | 51 (60.71%) | 37 (54.42%) | 26 (59.09%) |
| GC              | 30 (35.72%) | 25 (36.76%) | 15 (34.09%) |
| CC              | 3 (3.57%) | 6 (8.82%) | 3 (6.82%) |
| GG + GC        | 51 (60.71%) | 37 (54.42%) | 26 (59.09%) |
| CC              | 3 (3.57%) | 6 (8.82%) | 3 (6.82%) |
| G allele       | 132 (78.57%) | 99 (72.79%) | 67 (76.14%) |
| C allele       | 36 (21.43%) | 37 (27.21%) | 21 (23.86%) |

Analyzed by χ² tests, a intractable GD vs. GD in remission, b severe HD vs. mild HD, NS, not significant.
mild destruction of the thyroid because the Th1/Th2 ratio is lower in patients with mild HD than in those with severe HD [12].

In the case of the rs223828 SNP of the TARC/CCL17 gene, we hypothesized that the frequency of the T allele carrier was higher in patients with mild HD and GD in remission than in those with severe HD and intractable GD, respectively, as patients with the T allele show a higher expression level of the TARC/CCL17 gene [41] and may have a higher proportion of Th2 cells in the thy-

### Table 6  Genotype and allele frequencies of polymorphisms in Th2-related genes in patients with AITD and in control subjects

| Gene | Allele | Control | All patients with AITD | All patients with GD | All patients with HD |
|------|--------|---------|------------------------|----------------------|---------------------|
|       | CC     | 54 (55.67%) | 207 (53.62%) | NS* | 99 (53.23%) | 108 (54%) |
|       | CT     | 34 (35.05%) | 155 (40.16%) | 72 (38.71%) | 83 (41.5%) |
|       | TT     | 9 (9.28%) | 24 (6.22%) | 15 (8.06%) | 9 (4.5%) |
|       | CC     | 54 (55.67%) | 207 (53.62%) | NS* | 99 (53.23%) | 108 (54%) |
|       | CT + TT | 43 (44.33%) | 179 (46.38%) | NS* | 87 (46.77%) | 92 (46%) |
|       | CC + CT | 88 (90.72%) | 362 (93.78%) | NS* | 171 (91.94%) | 191 (95.5%) |
|       | TT     | 9 (9.28%) | 24 (6.22%) | 15 (8.06%) | 9 (4.5%) |
|       | C allele | 142 (73.2%) | 569 (73.7%) | NS* | 270 (72.58%) | 299 (74.75%) |
|       | T allele | 52 (26.8%) | 203 (26.3%) | NS* | 102 (27.42%) | 101 (25.25%) |
|       | IL25   |        |                  |                  |                  |
|       | C allele | 97 (50%) | 357 (46.01%) | NS | 173 (46.76%) | 184 (45.32%) |
|       | T allele | 97 (50%) | 419 (53.99%) | NS | 197 (53.24%) | 222 (54.68%) |
|       | STAT6  |        |                  |                  |                  |
|       | C allele | 64 (67.37%) | 233 (59.9%) | NS | 109 (58.6%) | 124 (61.08%) |
|       | T allele | 29 (30.53%) | 143 (36.76%) | NS | 68 (36.56%) | 75 (36.95%) |
|       | TT     | 2 (2.1%) | 13 (3.34%) | NS | 9 (4.84%) | 4 (1.97%) |
|       | CC     | 64 (67.37%) | 233 (59.9%) | NS | 109 (58.6%) | 124 (61.08%) |
|       | CT + TT | 31 (32.63%) | 156 (40.1%) | NS | 77 (41.4%) | 79 (38.92%) |
|       | CC + CT | 93 (97.9%) | 376 (96.66%) | NS | 177 (95.16%) | 199 (98.03%) |
|       | TT     | 2 (2.1%) | 13 (3.34%) | NS | 9 (4.84%) | 4 (1.97%) |
|       | C allele | 157 (82.63%) | 609 (78.28%) | NS | 286 (76.88%) | 323 (79.56%) |
|       | T allele | 33 (17.37%) | 169 (21.72%) | NS | 86 (23.12%) | 83 (20.44%) |
|       | STAT6  |        |                  |                  |                  |
|       | C allele | 22 (15.6%) | 61 (14.49%) | NS | 26 (12.15%) | 35 (16.91%) |
|       | T allele | 63 (44.68%) | 213 (50.59%) | NS | 115 (53.74%) | 98 (47.34%) |
|       | TT     | 56 (39.72%) | 147 (34.92%) | NS | 73 (34.11%) | 74 (35.75%) |
|       | CC     | 22 (15.6%) | 61 (14.49%) | NS | 26 (12.15%) | 35 (16.91%) |
|       | CT + TT | 119 (84.4%) | 360 (85.51%) | NS | 188 (87.85%) | 172 (83.09%) |
|       | CC + CT | 85 (60.28%) | 274 (65.08%) | NS | 141 (65.89%) | 133 (64.25%) |
|       | TT     | 56 (39.72%) | 147 (34.92%) | NS | 73 (34.11%) | 74 (35.75%) |
|       | C allele | 107 (37.94%) | 335 (39.70%) | NS | 167 (39.02%) | 168 (40.58%) |
|       | T allele | 175 (62.06%) | 507 (60.21%) | NS | 261 (60.98%) | 246 (59.42%) |

Analyzed by χ² tests, * vs. control, NS, not significant

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Table 7  Genotype and allele frequencies of the polymorphisms in Th2-related genes genotyped in this study in patients with HD, GD

| Genotype | control | in remission | intractable GD | HD | severe | mild |
|----------|---------|--------------|----------------|----|--------|------|
| STAT6 rs324011 | | | | | | |
| CC | 64 (67.37%) | 51 (58.62%) | 29 (61.7%) | 67 (68.37%) | 31 (60.79%) | | NS |
| CT | 29 (30.53%) | 31 (35.63%) | 17 (36.17%) | 28 (28.57%) | 19 (37.25%) | | NS |
| TT | 2 (2.1%) | 5 (5.75%) | 1 (2.13%) | 3 (3.06%) | 1 (1.96%) | | NS |

| STAT6 rs324015 | | | | | | |
| CC | 22 (15.6%) | 15 (13.89%) | 4 (7.27%) | 15 (15.31%) | 8 (15.68%) | | NS |
| CT | 63 (44.68%) | 51 (47.22%) | 36 (65.45%) | 46 (46.94%) | 20 (39.22%) | | NS |
| TT | 56 (39.72%) | 42 (38.89%) | 15 (27.28%) | 37 (37.75%) | 23 (45.1%) | | NS |

| TARC/CCL17 rs223828 | | | | | | |
| CC | 11 (11.34%) | 13 (14.94%) | 1 (2.13%) | 11 (11.22%) | 2 (3.92%) | | NS |
| CT | 75 (77.32%) | 61 (70.12%) | 36 (76.6%) | 70 (71.43%) | 39 (76.47%) | | NS |
| TT | 11 (11.34%) | 13 (14.94%) | 10 (21.27%) | 17 (17.35%) | 10 (19.61%) | | NS |

| IL25 rs7145531 | | | | | | |
| CC | 54 (55.67%) | 42 (48.28%) | 28 (58.33%) | 54 (55.67%) | 27 (55.1%) | | NS |
| CT | 34 (35.05%) | 37 (42.52%) | 18 (37.5%) | 39 (40.21%) | 21 (42.86%) | | NS |
| TT | 9 (9.28%) | 8 (9.2%) | 2 (4.17%) | 4 (4.12%) | 1 (2.04%) | | NS |

| C allele | 142 (73.2%) | 121 (69.54%) | 74 (77.08%) | 147 (75.77%) | 75 (76.53%) | | NS |
| T allele | 52 (26.8%) | 53 (30.46%) | 22 (22.92%) | 47 (24.23%) | 23 (24.37%) | | NS |

| CC + CT | 88 (90.72%) | 79 (90.8%) | 46 (95.83%) | 93 (95.88%) | 48 (97.96%) | | NS |
| TT | 9 (9.28%) | 8 (9.2%) | 2 (4.17%) | 4 (4.12%) | 1 (2.04%) | | NS |

| C allele | 97 (50%) | 87 (50%) | 38 (40.43%) | 92 (46.94%) | 43 (42.16%) | | NS |
| T allele | 97 (50%) | 87 (50%) | 56 (59.57%) | 104 (53.06%) | 59 (57.84%) | | NS |

| CC + TT | 86 (88.66%) | 74 (85.06%) | 46 (97.87%) | 87 (88.78%) | 49 (96.08%) | | NS |
| TT | 11 (11.34%) | 13 (14.94%) | 10 (21.27%) | 17 (17.35%) | 10 (19.61%) | | NS |

| C allele | 97 (50%) | 87 (50%) | 38 (40.43%) | 92 (46.94%) | 43 (42.16%) | | NS |
| T allele | 97 (50%) | 87 (50%) | 56 (59.57%) | 104 (53.06%) | 59 (57.84%) | | NS |

| CC | 64 (67.37%) | 51 (58.62%) | 29 (61.7%) | 67 (68.37%) | 31 (60.79%) | | NS |
| CT | 29 (30.53%) | 31 (35.63%) | 17 (36.17%) | 28 (28.57%) | 19 (37.25%) | | NS |
| TT | 2 (2.1%) | 5 (5.75%) | 1 (2.13%) | 3 (3.06%) | 1 (1.96%) | | NS |

| C allele | 157 (82.63%) | 133 (76.44%) | 75 (79.79%) | 162 (82.65%) | 81 (79.41%) | | NS |
| T allele | 33 (17.37%) | 41 (23.56%) | 19 (20.21%) | 34 (17.35%) | 21 (20.59%) | | NS |

| CC | 22 (15.6%) | 15 (13.89%) | 4 (7.27%) | 15 (15.31%) | 8 (15.68%) | | NS |
| CT | 63 (44.68%) | 51 (47.22%) | 36 (65.45%) | 46 (46.94%) | 20 (39.22%) | | NS |
| TT | 56 (39.72%) | 42 (38.89%) | 15 (27.28%) | 37 (37.75%) | 23 (45.1%) | | NS |

| C allele | 107 (37.94%) | 81 (37.5%) | 44 (40%) | 76 (38.78%) | 36 (35.29%) | | NS |
| T allele | 175 (62.06%) | 135 (62.5%) | 66 (60%) | 120 (61.22%) | 66 (64.71%) | | NS |

Analyzed by $\chi^2$ tests, *intractable GD vs. GD in remission, **severe HD vs. mild HD, NS, not significant.

Thyroid tissue. Expectedly, the frequency of the T carrier (CT + TT genotypes) was higher in patients with GD in remission than in those with intractable GD (Table 7). The frequency of T carrier (CT + TT genotypes) in patients with GD and intractable GD was similar in control subjects (Tables 6, 7). This suggested that the effect of this SNP on the development of GD may be minor. Therefore, we supposed that the T allele of this SNP may
be associated with the recruitment and migration of Th2 cells by producing TARC/CCL17 [24] and with the suppression of the differentiation and activation of Th17 [16, 18], which results in the suppression of the intractability of GD. On the other hand, we did not find any differences in allele frequency of this SNP between patients with severe HD and those with mild HD. This indicates that the effect of this SNP on the severity of HD may be minor.

In the case of the rs324011 and rs324015 SNPs of the STAT6 gene, although there was no significant difference in their frequencies among each patient’s group of both GD and HD, the frequency of the T allele carrier in both the rs324011 and rs324015 SNPs tended to be higher in patients with mild HD than in those with severe HD (Table 7). Therefore, we evaluated the expression levels of STAT6 mRNA to clarify the possibility of a difference in the STAT6 gene expression among the genotypes of these SNPs. However, we could not find any significant differences among each genotype of these SNPs and STAT6 mRNA expression. We suggest, therefore, that these SNPs may not be associated with the expression level of the STAT6 gene and the susceptibility and the prognosis of AITDs. Moreover, the rs7145531 SNP of the IL25 gene also may not be associated with the development of AITD because there was no significant difference in the distribution of genotypes among each patient’s group (Tables 6, 7).

In conclusion, the CRTH2 rs545659 SNP was associated with the susceptibility of GD. The CRTH2 rs545659 and rs634681 SNPs were associated with the severity of HD. The TARC/CCL17 rs223828 SNP was associated with the intractability of GD.

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Disclosure

The authors declare that they do not have any conflicts of interest.

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