RESEARCH ARTICLE

**HLA-DRB1 and DQB1 alleles in Japanese type 1 autoimmune hepatitis: The predisposing role of the DR4/DR8 heterozygous genotype**

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Abstract

**Objective**

Autoimmune hepatitis (AIH) is a chronic progressive liver disease. AIH is composed predominantly of type 1 in Japanese populations. The genetic and environmental factors are associated with the pathogenesis of AIH. **HLA-DRB1*03:01** and **DQB1*04:01** are associated with type 1 AIH in European and **DQB1*04:05** in Japanese populations. Here, we conducted an HLA association study in order to find HLA alleles or haplotypes predisposing or protective for Japanese AIH.

**Methods**

**HLA-DRB1 and DQB1 genotyping** of 360 type 1 AIH patients and 1026 healthy controls was performed.

**Results**

The predisposing association of **DRB1*04:01** (P = 0.0006, corrected PC = 0.0193, odds ratio [OR] 2.97, 95% confidence interval [CI] 1.62–5.43), **DQB1*04:05** (P = 1.89×10−21, PC = 5.86×10−20, OR 3.41, 95% CI 2.65–4.38), and **DQB1*04:01** (P = 4.66×10−18, PC = 6.99×10−17, OR 3.89, 95% CI 2.84–5.33) and the protective association of **DRB1*13:02** (P...
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**Conclusions**

The important roles of specific combinations of DRB1 and DQB1 alleles or haplotypes in the pathogenesis of type 1 AIH were suggested. The association of DRB4/DR8 heterozygous genotype suggested the pathologic importance of trans-complementing DQαβ heterodimer molecules encoded by DQA1 allele of one haplotype and the DQB1 allele of the other haplotype, as it was proposed in the HLA association studies of Type 1 diabetes.

**Introduction**

Autoimmune hepatitis (AIH) is a very rare chronic progressive liver disease with autoimmune features [1,2,3]. Type 1 AIH is characterized by the presence of serum anti-nuclear antibodies (ANA) or anti-smooth muscle antibodies (ASMA) and type 2 AIH by type 1 liver-kidney microsomal antibodies. AIH is composed predominantly of type 1 in Japanese populations. Although the disease etiology is uncertain, it is considered that the genetic and environmental factors are associated with the pathogenesis of AIH. Many studies including a recent genome-wide association study [4] showed the genetic association of AIH with genes located within human leukocyte antigen (HLA) region. HLA-DRB1*03:01 and *04:01 are associated with AIH in European populations [3]; *04:05 is associated in Japanese and Korean populations [6,7,8,9]. In addition, several studies have shown that DRB1*04:04, *04:05, and *13:01 are associated with AIH in Latin America [10,11,12,13]. DRB1*08 alleles are also reported to be associated with AIH in Indian and Iranian, but not in Pakistani populations [14,15,16]. On the other hands, DRB1*15:01 is protective for the susceptibility of AIH in European and Japanese populations [5,6]. DRB1*13:02, which differ by one amino acid residue from DRB1*13:01, is protectively associated with AIH in Latin America [11,13,17] and in Japan [8].

It was reported in the genome-wide association study that HLA is the sole strong genetic factor for the susceptibility of type 1 AIH [4]. The HLA region was scanned and the most important loci for the susceptibility of type 1 AIH was reported to be DRB1 [18]. It was suggested that no other genes in the HLA region are associated with type 1 AIH. However, DRB1 is in strong linkage disequilibrium with DQB1 and it is difficult to differentiate the role of DRB1 and DQB1 in the pathogenesis of type 1 AIH. Although HLA alleles are known to confer the risk for various autoimmune diseases, the precise mechanisms have not sufficiently been revealed. The risk alleles are different in these autoimmune diseases [19]. It was considered that different auto-antigens are presented by different disease-specific risk alleles; the presented auto-antigens are restricted by HLA alleles and are influenced by non-HLA genes, environmental factors, or precipitating events. The complex of auto-antigens and risk alleles...
stimulate self-reactive T cells, resulting in the eliciting of diseases [20]. In this study, we conducted an HLA association study in order to search HLA alleles or haplotypes predisposing or protective for Japanese AIH.

**Materials and methods**

**Patients and healthy controls**

Three hundred sixty type I AIH patients were enrolled from the register of Japanese National Hospital Organization Liver Registry [21]. The AIH patients without any other types of liver diseases satisfied the criteria of International Autoimmune Hepatitis Group (IAIHG) for diagnosis of type I AIH [22]. The healthy controls (n = 1026; mean age ± SD, 37.7 ± 11.7 years, 303 male [29.8%]) were recruited at Sagamihara Hospital, the University of Tokyo, Teikyo University, and Kanazawa University [23,24] or by the Pharma SNP Consortium (Tokyo, Japan) [25]. All the patients and the healthy individuals were native Japanese living in Japan. The study was reviewed and approved by University of Tsukuba Research Ethics Committee, Nagasaki University Research Ethics Committee, and the NHO central Institutional Review Board. Informed consents in writing were obtained from all the participants. The study was performed in accordance with the principles expressed in the Declaration of Helsinki.

**Genotyping methods**

Genotyping of HLA-DRB1 and DQB1 was conducted by the polymerase chain reaction with sequence-specific oligonucleotide probes (WAKFlow HLA typing kits, Wakunaga, Hiroshima, Japan), using the Bio-Plex 200 system (Bio-Rad, Hercules, CA). HLA-DR4 serological group includes DRB1*04:01, *04:03, *04:04, *04:05, *04:06, *04:07, and *04:10. DR6 is composed of DRB1*13:01, *13:02, *14:03, *14:04, *14:05, *14:06, *14:07, *14:29, and *14:54. DR8 consists of DRB1*08:02, *08:03, and *08:09. Genotyping results of HLA-DRB1 and DQB1 for some of the AIH patients were previously reported [8]. Genotyping results of DRB1 for all of the healthy controls (n = 1026) were previously reported [8,23,24]. Reported genotyping results of DQB1 for some of the healthy controls (n = 413; mean age ± SD, 39.3 ± 11.0 years, 61 male [14.8%]) were used for the analyses on DQB1 allele, DQB1 genotype, DRB1-DQB1 haplotype, DRB1-DQB1 diplotype, and acid residues in the DQβ chain [24]. DRB1-DQB1 haplotypes were elucidated by direct counting, because DRB1 is in strong linkage disequilibrium with DQB1.

**Statistical analysis**

Differences of AIH characteristics were analyzed by Mann-Whitney’s U test or Fisher’s exact test using 2x2 contingency tables. Association of allele carrier frequencies, haplotype carrier frequencies, or amino acid residue carrier frequencies was analyzed by Fisher’s exact test using 2x2 contingency tables under the dominant model. Differences of genotype frequencies or diplotype (the specific combination of DRB1-DQB1 haplotypes) frequencies were analyzed by Fisher’s exact test using 2x2 contingency tables. Adjustment for multiple comparisons was conducted with Bonferroni method; corrected P (Pc) values were calculated by multiplying the P value by the number of alleles or amino acid residues tested.

**Results**

**Clinical features of type I AIH patients**

Characteristics of the type I AIH patients are shown in Table 1. Among 360 AIH patients, 314 (87.2%) were positive for ANA, 118 (38.6%) were positive for ASMA. Of overall AIH, 227 (63.1%) were definite AIH.
To compare HLA-DRB1 allele carrier frequency of the AIH patients and the healthy controls, we performed HLA-DRB1 genotyping (Table 2). A significant association between type I AIH and DRB1:04:05 (P = 1.89×10$^{-21}$, corrected P [Pc] = 5.86×10$^{-20}$, odds ratio [OR] 3.41, 95% confidence interval [CI] 2.65–4.38) was detected. DRB1:04:01 was also associated with type I AIH (P = 0.0006, Pc = 0.0193, OR 2.97, 95% CI 1.62–5.43). On the contrary, DRB1:13:02 was found to be protectively associated with type I AIH (P = 0.0003, Pc = 0.0080, OR 0.48, 95% CI 0.32–0.72). HLA-DR4 serological group was associated with type I AIH (P = 3.84×10$^{-18}$, OR 2.98, 95% CI 2.32–3.83), but DR6 is protectively associated (P = 2.10×10$^{-5}$, OR 0.54, 95% CI 0.41–0.72). Thus, DRB1:04:05 and DRB1:13:02 were predisposing and DRB1:13:02 was protective for AIH.

Demographic features of type I AIH patients with or without DRB1:04:05 or *13:02 were compared (Table 3). Serum levels of Immunoglobulin G (IgG), Immunoglobulin M (IgM), and IAIHG score were higher in AIH patients with DRB1:04:05 than without. Positive rate of ASMA and the rate of definite AIH were higher in AIH patients with DRB1:04:05 than without. The complication rate of cirrhosis tended to be higher in AIH patients with DRB1:13:02 than without. Thus, specific clinical features of AIH patients possessing DRB1:04:05 were observed.

**HLA-DRB1 genotype in type I AIH**

We investigated the genotype frequency in the AIH patients (Table 4). The homozygosity for DRB1:04:05 (OR 2.79, 95% CI 1.45–5.38) did not confer higher OR for AIH than heterozygosity for DRB1:04:05 (OR 3.10, 95% CI 2.40–4.00). In contrast, the homozygosity for DRB1:13:02 (OR 0.15, 95% CI 0.01–2.56) conferred lower OR than heterozygosity for DRB1:13:02 (OR 0.51, 95%
CI 0.34–0.78). The frequency of the DRB1*04:05/*13:02 genotype was comparable. Of interest, higher frequencies of DRB1*04:05/*08:02 (P = 3.78X10^{-6}, OR 6.70, 95% CI 2.89–15.54) and DRB1*04:05/*08:03 (P = 9.80X10^{-7}, OR 4.54, 95% CI 2.47–8.35) genotypes in AIH were observed. Similarly, the DR4/DR8 genotype frequency in AIH was markedly increased (P = 3.12X10^{-9}, OR 3.52, 95% CI 2.34–5.29). Thus, some specific heterozygous genotypes were predisposing for AIH.

Certain amino acid residues in HLA-DRβ chains were associated with AIH

The association with AIH with respect to each amino acid residue in the HLA-DRβ chain was analyzed. The amino acid residues of 11V, 13H, 33H, 57S, and 96Y in the DRβ chain showed

Table 2. HLA-DRB1 allele carrier frequency in the AIH patients and healthy controls.

| Allele          | Case (n = 360) | Control (n = 1026) | P     | OR   | P<sub>c</sub> | 95%CI       |
|-----------------|---------------|--------------------|-------|------|--------------|-------------|
| DRB1*01:01     | 26 (7.2)      | 110 (10.7)         | 0.0634| 0.65 | NS           | (0.42–1.01) |
| DRB1*03:01     | 1 (0.3)       | 3 (0.3)            | 1.0000| 0.95 | NS           | (0.10–9.16) |
| DRB1*04:01     | 22 (6.1)      | 22 (2.1)           | 0.0006| 2.97 | 0.0193       | (1.62–5.43) |
| DRB1*04:03     | 15 (4.2)      | 47 (4.6)           | 0.8823| 0.91 | NS           | (0.50–1.64) |
| DRB1*04:04     | 0 (0.0)       | 4 (0.4)            | 0.5780| 0.32 | NS           | (0.02–5.87) |
| DRB1*04:05     | 185 (51.4)    | 243 (23.7)         | 1.89X10^{-21} | 3.41 | 5.68X10^{-20} | (2.65–4.38) |
| DRB1*04:06     | 15 (4.2)      | 76 (7.4)           | 0.0351| 0.54 | NS           | (0.31–0.96) |
| DRB1*04:07     | 5 (1.4)       | 15 (1.5)           | 1.0000| 0.95 | NS           | (0.34–2.63) |
| DRB1*04:10     | 12 (3.3)      | 32 (3.1)           | 0.8616| 1.07 | NS           | (0.55–2.10) |
| DRB1*07:01     | 2 (0.6)       | 9 (0.9)            | 0.7382| 0.63 | NS           | (0.14–2.94) |
| DRB1*08:02     | 35 (9.7)      | 72 (7.0)           | 0.1080| 1.43 | NS           | (0.93–2.18) |
| DRB1*08:03     | 58 (16.1)     | 153 (14.9)         | 0.6091| 1.10 | NS           | (0.79–1.52) |
| DRB1*08:09     | 0 (0.0)       | 2 (0.2)            | 1.0000| 0.57 | NS           | (0.03–11.87)|
| DRB1*09:01     | 77 (21.4)     | 280 (27.3)         | 0.0298| 0.72 | 0.8949       | (0.54–0.97) |
| DRB1*10:01     | 5 (1.4)       | 5 (0.5)            | 0.1381| 2.88 | NS           | (0.83–9.99) |
| DRB1*11:01     | 8 (2.2)       | 41 (4.0)           | 0.1361| 0.55 | NS           | (0.25–1.18) |
| DRB1*12:01     | 25 (6.9)      | 75 (7.3)           | 0.9059| 0.95 | NS           | (0.59–1.51) |
| DRB1*12:02     | 11 (3.1)      | 37 (3.6)           | 0.7384| 0.84 | NS           | (0.43–1.67) |
| DRB1*13:01     | 2 (0.6)       | 8 (0.8)            | 1.0000| 0.71 | NS           | (0.15–3.36) |
| DRB1*13:02     | 30 (8.3)      | 163 (15.9)         | 0.0003| 0.48 | 0.0080       | (0.32–0.72) |
| DRB1*14:02     | 1 (0.3)       | 0 (0.0)            | 0.2597| 8.57 | NS           | (0.35–210.76)|
| DRB1*14:03     | 5 (1.4)       | 44 (4.3)           | 0.0078| 0.31 | 0.2337       | (0.12–0.80) |
| DRB1*14:04     | 0 (0.0)       | 4 (0.4)            | 0.5780| 0.32 | NS           | (0.02–5.67) |
| DRB1*14:05     | 13 (3.6)      | 40 (3.9)           | 0.8744| 0.92 | NS           | (0.49–1.75) |
| DRB1*14:06     | 5 (1.4)       | 29 (2.8)           | 0.1655| 0.48 | NS           | (0.19–1.26) |
| DRB1*14:07     | 1 (0.3)       | 2 (0.2)            | 1.0000| 1.43 | NS           | (0.13–15.78)|
| DRB1*14:54     | 20 (5.6)      | 58 (5.7)           | 1.0000| 0.98 | NS           | (0.58–1.66) |
| DRB1*15:01     | 41 (11.4)     | 139 (13.5)         | 0.3171| 0.82 | NS           | (0.57–1.19) |
| DRB1*15:02     | 62 (17.2)     | 224 (21.8)         | 0.0692| 0.74 | NS           | (0.55–1.02) |
| DRB1*16:02     | 5 (1.4)       | 18 (1.8)           | 0.8119| 0.79 | NS           | (0.29–2.14) |
| DR4            | 238 (66.1)    | 406 (39.6)         | 3.84X10^{-18} | 2.98 | 2.32X10^{-3} | (2.32–3.83) |
| DR6 (*13, *14) | 74 (20.6)     | 332 (32.4)         | 2.10X10^{-5} | 0.54 | (0.41–0.72)  |
| DR8            | 92 (25.6)     | 220 (21.4)         | 0.1234| 1.26 | (0.95–1.66)  |
Table 3. Comparison of the demographics between AIH patients with or without DRB1*04:05 or * 13:02.

|               | DRB1*04:05(+) | DRB1*04:05(-) | P      | DRB1*13:02(+) | DRB1*13:02(-) | P     |
|---------------|---------------|---------------|--------|---------------|---------------|-------|
| Number        | 185           | 175           |        | 30            | 330           |       |
| Male, n (%)   | 20 (10.8%)    | 23 (13.1%)    | *0.519 | 2 (6.7%)      | 41 (12.4%)    | *0.5559 |
| Age at onset, years (SD) | 58.1 (±14.3) | 59.2 (±14.7) | 0.2897 | 59.5 (±15.8) | 58.5 (±14.4) | 0.6174 |
| Mean age, years (SD) | 63.2 (±12.2) | 62.6 (±14.8) | 0.6877 | 62.0 (±15.9) | 63.0 (±13.3) | 0.9408 |
| Albumin (g/dl) (SD) | 3.7 (±0.7)   | 3.8 (±0.9)    | 0.2699 | 3.7 (±0.6)   | 3.8 (±0.8)    | 0.2744 |
| Total bilirubin (mg/dl) (SD) | 3.6 (±4.6)   | 3.8 (±5.2)    | 0.8023 | 4.4 (±5.6)   | 3.6 (±4.9)    | 0.2202 |
| ALT(IU/L) (SD) | 428.5 (±409.7) | 513.2 (±659.0) | 0.5735 | 498.4 (±498.4) | 467.1 (±550.9) | 0.7813 |
| AST(IU/L) (SD) | 488.1 (±492.3) | 525.1 (±529.0) | 0.7793 | 549.4 (±562.2) | 502.2 (±505.8) | 0.8568 |
| ALP(IU/L) (SD) | 450.3 (±185.0) | 478.4 (±237.1) | 0.6656 | 463.8 (±217.1) | 464.0 (±212.0) | 0.9474 |
| IgG (mg/dl) (SD) | 2587.1 (±1005.7) | 2119.8 (±840.8) | 2.10X10⁻⁶ | 2242.9 (±878.5) | 2370.6 (±964.3) | 0.3771 |
| Platelets (10⁴/μl) (SD) | 18.5 (±7.1) | 18.5 (±7.5) | 0.9008 | 18.1 (±6.6) | 18.5 (±7.4) | 0.8625 |
| ANA ≥ 1:40, n (%) | 164 (88.6%) | 150 (85.7%) | *0.4328 | 29 (96.7%) | 285 (86.4%) | *0.1510 |
| ASMA ≥ 1:40, n (%) | 91 (55.2%) | 27 (19.1%) | *6.46X10⁻¹¹ | 7 (33.3%) | 111 (38.9%) | *0.6515 |
| Cirrhosis, n (%) | 26 (14.1%) | 23 (13.1%) | *0.8782 | 8 (26.7%) | 41 (12.4%) | *0.0462 |
| IAIHG score (SD) | 16.9 (3.1%) | 15.6 (2.9%) | 1.54X10⁻⁵ | 15.7 (2.9%) | 16.3 (3.1%) | 0.3476 |
| Definite AIH, n (%) | 133 (71.9%) | 94 (53.7%) | *0.0005 | 17 (56.7%) | 210 (63.6%) | *0.4383 |

Association was tested between AIH patients with or without DRB1*04:05 or * 13:02 by Fisher’s exact test using 2x2 contingency tables or Mann-Whitney’s U test.

* Fisher’s exact test was employed.

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associations with AIH (Fig 1). Thus, this association analysis suggested roles for specific amino acid residues in the HLA-DRβ chain.

**HLA-DQB1 in type I AIH**

We next tried to compare HLA-DQB1 allele carrier frequency of the AIH patients with 413 of the 1026 healthy controls, since previously reported genotyping results of DQB1 were available for the 413 healthy controls [24]. When DRB1 genotyping results for the 413 healthy controls were compared with those of the AIH patients, similar tendencies were observed (S1 and S2 Tables). DQB1*04:01 allele was strongly associated with AIH (P = 4.66x10⁻¹⁸, P< 6.99x10⁻¹⁷, OR 3.89, 95% CI 2.84–5.33, S3 Table). We further examined HLA-DQB1 genotype (S4 Table). The homozygosity for DQB1*04:01 (OR 4.04, 95% CI 1.48–11.08) conferred comparative OR compared with heterozygosity for DQB1*04:01 (OR 3.47, 95% CI 2.52–4.77). The higher frequency of DQB1*04:01/*01:06:01 genotype in AIH was observed (P = 8.75X10⁻⁶, OR 3.24, 95% CI 1.89–5.56). Thus, some of DQB1 alleles or genotypes were predisposing for AIH.

**DRB1-DQB1 haplotype in type I AIH**

DRB1-DQB1 haplotype carrier frequencies were compared between the AIH patients and the 413 healthy controls (Table 5). Higher carrier frequencies of DRB1*04:01-DQB1*03:01 (P = 0.0007, OR 4.42 95% CI 1.77–11.01) and DRB1*04:05-DQB1*04:01 (P = 1.99x10⁻²⁰, OR 4.32, 95% CI 3.14–5.96) were found in the AIH patients. DRB1-DQB1 diplotype frequencies were also compared between the AIH patients and the 413 healthy controls (Table 5). The homozygosity for DRB1*04:05-DQB1*04:01 (OR 5.07, 95% CI 1.69–15.20) conferred slightly higher OR for AIH than heterozygosity for DRB1*04:05-DQB1*04:01 (OR 3.81, 95% CI 2.76–5.28). The diplotype frequencies of DRB1*04:05-DQB1*04:01/DRB1*08:02-DQB1*03:02...
Table 4. HLA-DRB1 genotype frequency in the AIH patients and controls.

|        | Case (n = 360) | Control (n = 1026) | P          | OR   | 95%CI   |
|--------|----------------|--------------------|------------|------|---------|
| *04:05/not *04:05 | 167 (46.4)    | 224 (21.8)        | 5.72X10^{-18} | 3.10 | (2.40–4.00) |
| *13:02/not *13:02  | 30 (8.3)       | 154 (15.0)        | 0.0011     | 0.51 | (0.34–0.78) |
| *04:01/not *04:01  | 22 (6.1)       | 21 (2.0)          | 0.0003     | 3.11 | (1.69–5.74) |
| *04:01/*04:05     | 4 (1.1)        | 1 (0.1)           | 0.0178     | 11.52 | (1.28–103.39) |
| *04:05/*04:05     | 18 (5.0)       | 19 (1.9)          | 0.0035     | 2.79 | (1.45–5.38) |
| *04:05/*08:02     | 18 (5.0)       | 8 (0.8)           | 3.78X10^{-6} | 6.70 | (2.89–15.54) |
| *04:05/*08:03     | 27 (7.5)       | 18 (1.8)          | 9.80X10^{-7} | 4.54 | (2.47–8.35) |
| *04:05/*13:02     | 8 (2.2)        | 25 (2.4)          | 1.0000     | 0.91 | (0.41–2.04) |
| *13:02/*13:02     | 0 (0.0)        | 9 (0.9)           | 0.1225     | 0.15 | (0.01–2.56) |
| DR4/DR4           | 34 (9.4)       | 57 (5.6)          | 0.0132     | 1.77 | (1.14–2.76) |
| DR8/DR8           | 5 (1.4)        | 16 (1.6)          | 1.0000     | 0.89 | (0.32–2.44) |
| DR6/DR6           | 3 (0.8)        | 25 (2.4)          | 0.0796     | 0.34 | (0.10–1.12) |
| DR4/DR8           | 54 (15.0)      | 49 (4.8)          | 3.12X10^{-9} | 3.52 | (2.34–5.29) |
| DR4/DR6           | 32 (8.9)       | 90 (8.8)          | 0.9144     | 1.01 | (0.66–1.55) |

AIH: autoimmune hepatitis, OR: odds ratio, 95%CI: confidence interval. Genotype frequencies are shown in parenthesis (%). Association was tested by Fisher’s exact test using 2X2 contingency tables.

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(P = 0.0004, OR 24.77, 95% CI 1.45–424.31) and DRB1*04:05-DQB1*04:01/DRB1*08:03-DQB1*06:01 (P = 1.18X10^{-6}, OR 10.64, 95% CI 3.19–35.46) were higher in AIH patients, suggesting the predisposing role of some specific heterozygous diplotypes in AIH.

Certain amino acid residues in HLA-DQβ chains were associated with AIH

The association with AIH with respect to each amino acid residue in the HLA-DQβ chain was analyzed in the comparison with the 413 healthy controls. The amino acid residues of 23L, 56L, 70E, and 71D in the DQβ chain showed associations with AIH (Fig 2). When each amino acid residue frequency in the DRβ chain for the 413 healthy controls was compared with that of the AIH patients, similar tendencies were observed (S1 Fig). Thus, this association analysis suggested roles for specific amino acid residues in the HLA-DQβ chains.

Discussion

Several studies have reported that type 1 AIH is associated with HLA-DRB1*03:01 and DRB1*04:01 in European [5] and DRB1*04:05 in Japanese populations (Fig 3) [6,7,8]. In the present study, we showed an association of Japanese AIH with DRB1*04:01 and *04:05, indicating the common predisposing DRB1*04:01 allele for AIH between European and Japanese populations. DRB1*04:05 is also common predisposing allele for AIH between Latin America [13] and Japan. In previous studies, DRBI*13:02 was protectively associated with type 1 AIH in Latin America [11,13,17]. We also confirmed a protective association of DRB1*13:02 with Japanese AIH [8], but could not replicate the protective effects of DRB1*15:01 [5,6]. These data indicated that the common protective DRB1*13:02 allele for AIH between Latin America and Japan is also the protective allele shared by multiple autoimmune diseases [19].

Specific demographic features of Japanese AIH patients with DRB1*04:05 were observed (Table 3). Elevated serum levels of IgG and IgM were detected in AIH patients with DRB1*04:05, as it was previously described [7]. In the present study, the IAIHG score, the positive rate of ASMA, and the rate of definite AIH were newly found to be higher in Japanese AIH patients.
with DRB1*04:05. Although HLA alleles are known to confer the risk for various autoimmune diseases, the precise mechanisms have not sufficiently been revealed. Thus, Japanese AIH patients with DRB1*04:05 have typical clinical traits, probably because the auto-antigens

![Fig 1. Associations of amino acid residues in DRβ chain with AIH. Each amino acid residue frequency in the HLA-DRβ chain for the 1026 healthy controls was compared with that of the AIH patients. Differences of amino acid residue carrier frequencies were analyzed by Fisher’s exact test using 2x2 contingency tables. Corrected P (Pc) values were calculated by multiplying the P value by the number of amino acid residues tested. Predisposing associations were indicated by filled circles and protective associations by open circles.](https://doi.org/10.1371/journal.pone.0187325.g001)

Table 5. **DRB1-DQB1** haplotype carrier or diplotype frequency in the AIH patients and controls.

| **DRB1-DQB1** haplotype | Case (n = 360) | Control (n = 413) | P     | OR       | 95%CI      |
|-------------------------|---------------|-------------------|-------|----------|------------|
| *04:01-*03:01           | 22 (6.1)      | 6 (1.5)           | 0.0007| 4.42     | (1.77–11.01)|
| *04:05-*04:01           | 182 (50.6)    | 79 (19.1)         | 1.99X10^{-20} | 4.32     | (3.14–5.96) |
| *08:02-*03:02           | 21 (5.8)      | 21 (5.1)          | 0.7508| 1.16     | (0.62–2.15) |
| *08:02-*04:02           | 13 (3.6)      | 16 (3.9)          | 1.0000| 0.93     | (0.44–1.96) |
| *08:03-*03:01           | 3 (0.8)       | 2 (0.5)           | 0.6682| 1.73     | (0.29–10.39)|
| *08:03-*06:01           | 57 (15.8)     | 57 (13.8)         | 0.4768| 1.17     | (0.79–1.75) |
| *13:02-*06:04           | 31 (8.6)      | 49 (11.9)         | 0.1559| 0.70     | (0.44–1.12) |

**DRB1-DQB1** diplotype

| *04:05-*04:01/04:01     | 165 (45.8)    | 75 (18.2)         | 1.17X10^{-16} | 3.81     | (2.76–5.28) |
| *04:05-*04:01/04:03:01  | 4 (1.1)       | 1 (0.2)           | 0.1898| 4.63     | (0.52–41.61) |
| *04:05-*04:01/04:05:04:01| 17 (4.7)     | 4 (1.0)           | 0.0015| 5.07     | (1.69–15.20) |
| *04:05-*04:01/08:02:03:02| 10 (2.8)     | 0 (0.0)           | 0.0004| 24.77    | (1.45–424.31)|
| *04:05-*04:01/08:02:04:02| 7 (1.9)      | 3 (0.7)           | 0.2017| 2.71     | (0.70–10.56) |
| *04:05-*04:01/08:03:03:01| 1 (0.3)      | 0 (0.0)           | 0.4657| 3.45     | (0.14–84.97) |
| *04:05-*04:01/08:03:06:01| 26 (7.2)     | 3 (0.7)           | 1.18X10^{-6}  | 10.64   | (3.19–35.46) |
| *04:05-*04:01/13:02:06:04| 8 (2.2)      | 8 (1.9)           | 0.8052| 1.15     | (0.43–3.10) |

AIH: autoimmune hepatitis, OR: odds ratio, 95%CI: confidence interval. Genotype frequencies are shown in parenthesis (%). Association was tested by Fisher’s exact test using 2X2 contingency tables.

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presented by DRB1*04:05 molecules would be important for development of the typical clinical traits of AIH.

It is well known that DRB1*04:01 in European [26,27] and DRB1*04:05 in Japanese populations [23,28] are associated with the susceptibility for rheumatoid arthritis (RA) and type 1 diabetes, in an analogous fashion to AIH. RA is a systemic autoimmune disease that affects synovial joints. RA-susceptible DRB1 alleles shared a conserved amino acid sequence at position 70–74 (QKRAA, RRRAA, or QRRAA) in HLA-DRβ chain and were designated as shared epitope alleles [23,26]. The shared epitope alleles include DRB1*01:01, *04:01, *04:04, *04:05, *04:10, *10:01, *14:02, and *14:06. However, neither DRB1*01:01 nor *04:10 seems to be a risk allele for AIH (Table 2). In the associations of DRB1 alleles with susceptibility to RA, a gene dosage effect was reported; homozygosity for predisposing DRB1 alleles confers higher OR than heterozygosity. However, we could not find any gene dosage effects of predisposing alleles or haplotypes in AIH. These data suggested the differential roles of DRB1 in the pathogenesis between AIH and RA.

Type 1 diabetes is an autoimmune disease that affects pancreatic β cells producing insulin, resulting in the dysregulation of glucose metabolism. Susceptible DRB1 alleles for type 1 diabetes are DRB1*03:01, *04:01, *04:02, *04:04, *04:05, and *08:01 and protective alleles are DRB1*15:01, *14:01, and *07:01 in European populations [27]. In Japanese populations, DRB1*04:05, *08:02, and *09:01 are predisposing alleles for type 1 diabetes and DRB1*15:02 is a protective allele [28]. No gene dosage effect was observed for DRB1*04:05, though a gene dosage effect for DRB1*09:01 was detected for type 1 diabetes. Similarly, we did not detect any gene dosage effects of the predisposing DRB1 alleles in DR8 genotypes in type 1 diabetes. In addition, higher frequencies of the DR3/DR4, DR4/DR4, and DR4/DR8 genotypes in type 1 diabetes were reported [27]. In an analogous fashion, frequencies of the DR4/DR8 genotypes were higher in Japanese type 1 AIH (Table 4). Since the allele frequency of DR4 is higher than that of DR8 in Japanese populations, DR4 is a risk allele by itself, but DR8 is not, and type 1 AIH is a

![Fig 2. Associations of amino acid residues in DQβ chain with AIH.](https://doi.org/10.1371/journal.pone.0187325.g002)
multifactorial disease, the DR4/DR8 genotype could not mainly contribute to the pathogenesis of type 1 AIH. It was also reported that the association of DRB1*08 with the susceptibility of type 1 AIH was not detected in European populations [29], because of low frequency of DRB1*08. The DR4/DR8 heterozygous genotypes may cause an increased probability of self-antigen presentation, resulting in the increased risk of the diseases. Thus, the manner of DRB1 association in type 1 AIH appears to be similar to that in type 1 diabetes.

Fig 3. Summary of the HLA-DRB1 alleles associated with type 1 AIH. The HLA-DRB1 alleles associated with type 1 AIH in European, Japanese, and Hispanic populations are illustrated. The underlined alleles are protective alleles.

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Augmented frequencies of the DR3/DR4 and DR4/DR8 heterozygous genotypes in type 1 diabetes was explained by the pathologic importance of trans-complementing DQα-β heterodimer molecules encoded by the DQA1 allele of one haplotype and the DQB1 allele of the other haplotype. The low stability of these molecules in trans was proposed to be causative to type 1 diabetes [27,30]. Analogously, DR4/DR8 heterozygous genotype was increased in Japanese type 1 AIH (Table 4), suggesting that trans-complementing DQα-β heterodimer molecules might also play a role in AIH. DRB1-DQB1 diplotyp analysis revealed that DRB1*04:05-DQB1*04:01/DRB1*08:02-DQB1*03:02 and DRB1*04:05-DQB1*04:01/DRB1*08:03-DQB1*06:01 were significantly associated with AIH (Table 5). Japanese type 1 AIH was not significantly associated with DRB1*08:02 or *08:03 (Table 2). Neither DQB1*03:02 nor *06:01 was associated with type 1 AIH (S1 Table). Based on the conserved haplotype structure in the Japanese population, the DQA1 allele in the haplotype of DRB1*04:05-DQB1*04:01 is presumed to be DQA1*03:03 [31] and the DQA1 alleles in DRB1*08:02-DQB1*03:02 and DRB1*08:03-DQB1*06:01 are estimated to be DQA1*03:01 and *01:03, respectively. The high risk diplotype DRB1*04:05-DQB1*04:01/DRB1*08:02-DQB1*03:02 is considered to encode DQA1*03:03-DQB1*04:01 and DQA1*03:01-DQB1*03:02 molecules in cis (DQα-β heterodimer molecules formed by the protein products of DQA1 and the DQB1 alleles from the same chromosome) and DQA1*03:03-DQB1*03:02 and DQA1*03:01-DQB1*04:01 molecules in trans (DQα-β heterodimer molecules formed by the protein products of DQA1 and the DQB1 alleles from the opposite chromosomes). The stabilities of these four types of DQα-β heterodimer molecules were estimated to be low, according to the previous study [30]. The other high risk diplotype DRB1*04:05-DQB1*04:01/DRB1*08:03-DQB1*06:01 is thought to encode DQA1*03:03-DQB1*04:01 and DQA1*01:03-DQB1*06:01 molecules in cis and DQA1*03:03-DQB1*06:01 and DQA1*01:03-DQB1*04:01 molecules in trans. The stabilities of these molecules except DQA1*01:03-DQB1*06:01 in cis were also estimated to be low [30]. In patients with the risk diplotype DRB1*04:05-DQB1*04:01/DRB1*08:03-DQB1*06:01, the low stability of trans-complementing DQα-β heterodimer molecules could explain the pathogenesis of type 1 AIH. In the case of type 1 diabetes, each of DRB1, DQA1, and DQB1 is believed to have independent genetic contribution in the disease susceptibility based on the data from haplotype analysis [27]. Similar scenario might also apply to AIH. However, such analysis could not be performed in this study, because of the limited variety of DRB1-DQB1 haplotypes in Japanese populations (Table 5). Furthermore, other culprit genes in linkage disequilibrium with DRB1-DQB1 loci might be causative for AIH. Thus, the results of the association analyses of DRB1 and DQB1 in type 1 AIH could propose several lines of explanations on the mechanisms underlined in the pathogenesis.

We detected that amino acid residues of 11V, 13H, 33H, 57S, and 96Y in the HLA-DRβ chain were associated with AIH (Fig 1A); these amino acids were encoded by DRB1*04:05 allele. It was also found that some amino acid residues of the DQβ chains were associated with type 1 AIH (Fig 1B). These amino acid residues were also encoded by DQB1*04:01. These data were influenced by the strongest predisposing haplotype DRB1*04:05-DQB1*04:01 for AIH, confirming the dominance of the DRB1*04:05-DQB1*04:01 haplotype in type 1 AIH in Japanese populations.

In conclusion, we showed the predisposing association of DRB1*04:01, DRB1*04:05, and DQB1*04:01 and the protective association of DRB1*13:02 with Japanese type 1 AIH. The association of DR4/DR8 heterozygous genotype with AIH was newly noted. With respect to DRB1-DQB1 haplotypes, DRB1*04:01-DQB1*03:01 and DRB1*04:05-DQB1*04:01 haplotypes were found to be associated with type 1 AIH. Of interest, the association of DRB1*04:05-DQB1*04:01/DRB1*08:02-DQB1*03:02 and DRB1*04:05-DQB1*04:01/DRB1*08:03-DQB1*06:01 diplotypes was revealed. These data suggested the roles of specific combinations of DRB1 and DQB1 alleles or haplotypes in the pathogenesis of type 1 AIH. Further large scale studies
should be performed to confirm these findings. In addition, because the HLA allele distribution pattern is different in other ethnic populations, it would be intriguing and informative to analyze DRB1 and DQB1 alleles in type 1 AIH in other populations.

Supporting information

S1 Fig. Associations of amino acid residues in DRβ chain with AIH. (PDF)

S1 Table. HLA-DRB1 allele carrier frequency in the AIH patients and the 413 healthy controls. (PDF)

S2 Table. HLA-DRB1 genotype frequency in the AIH patients and the 413 healthy controls. (PDF)

S3 Table. HLA-DQB1 allele carrier frequency in the AIH patients and the 413 healthy controls. (PDF)

S4 Table. HLA-DQB1 genotype frequency in the AIH patients and the 413 healthy controls. (PDF)

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