Sex Alters the MHC Class I HLA-A Association With Polyglandular Autoimmunity

Brigitte K. Flesch,¹* Jochem König,²* Lara Frommer,³ Martin P. Hansen,³ and George J. Kahaly³

¹Laboratory of Immunogenetics/HLA, German Red Cross Blood Service West, 55543 Bad Kreuznach and 58097 Hagen, Germany; ²Institute of Medical Biostatistics, Epidemiology and Informatics, Johannes Gutenberg University Medical Center, 55131 Mainz, Germany; and ³Molecular Thyroid Research Laboratory, Johannes Gutenberg University Medical Center, 55131 Mainz, Germany

ORCID numbers: 0000-0003-0441-430X (G. J. Kahaly).

Context: The major histocompatibility complex (MHC) strongly contributes to the development of polyglandular autoimmunity (PGA).

Objective: To evaluate the impact of sex on human leukocyte antigen (HLA) association with PGA for the first time.

Design: Cross-sectional immunogenetic study.

Setting: Academic tertiary referral Orphan Disease Center for PGA (ORPHA 282196) and immunogenetics laboratory.

Subjects: Patients (158) with coexistent type 1 diabetes and autoimmune thyroid disease (adult type 3 PGA, ORPHA 227982) and 479 unrelated healthy controls.

Interventions: All 637 white subjects were typed for HLA-A, -B, -DRB1, -DQA1, and -DQB1 alleles at a two-field level.

Main Outcome Measures: Modification of the gene-disease association by sex.

Results: MHC class I HLA-A association was sex related to both the total white adult type 3 PGA collective (n = 158, P = 0.0065), as well as in PGA patients with autoimmune Hashimoto thyroiditis (n = 91, P = 0.010). Compared with HLA-A*02:01, A*11:01 was over-represented in male patients, yet under-represented in women (OR 1.49, 95% CI 0.55 to 3.88 vs 0.42, 0.12 to 1.17). A*24:02 was under-represented in male but not in female patients (OR 0.37, 95% CI 0.11 to 1.04 vs 1.19, 0.65 to 2.15).

With the exclusion of the five most frequent alleles (A*01:01, A*02:01, A*03:01, A*11:01, and A*24:02), the sum of all other identified alleles was under-represented in male patients (OR 0.37, 0.18 to 0.72, P = 0.0046). The strong MHC HLA-B association with PGA (P < 0.0001) was not sex related (P = 0.55). Furthermore, no interaction with sex was observed for the MHC class II HLA-DRB1, -DQA1, and -DQB1 alleles.

Conclusion: MHC class I HLA-A association with type 3 PGA is significantly affected by sex. (J Clin Endocrinol Metab 104: 1680–1686, 2019)
Autoimmune polyendocrinopathy (ORPHA 282196) or polyglandular autoimmunity (PGA) is a rare disease (1), characterized by the coexistence of at least two autoimmune-induced endocrine disorders (2–6). The coexistence of autoimmune thyroid disease (AITD) and type 1 diabetes (T1D) is defined as the adult type 3 PGA syndrome type 3 variant (ORPHA 227982) (2–4, 7). PGA is inherited as an autosomal-dominant form with an incomplete penetrance and a high prevalence in already affected families, which demonstrates the relevance of genetic background (8). Indeed, the major histocompatibility complex (MHC) on chromosome 6 is important for both the pathomechanism of PGA, as well as being a major susceptibility gene for T1D and AITD (9–12). This also holds true for AITD (13, 14). HLA class II alleles trigger (auto-) immune responses via the presentation of exogenous protein peptides to CD4+ T cells (15), and DRB1*03:01, DRB1*04:01, DQA1*03:01, DQA1*05:01, DQB1*02:01, and DQB1*03:02 have been associated with PGA (12, 16–19).

HLA class I interacts with the immune response via the presentation of intracellular peptides to CD8+ T cells (20). The class I HLA-A2 allele seems to be protective against Graves disease (GD) but not against Hashimoto thyroiditis (HT) (21). In HT, the frequency of peripheral thyroperoxidase- and thyroglobulin-specific CD8+ T cells is significantly higher in HLA-A2-positive patients than in HLA-A2 negatives (22). In comparison, HLA-B*57:01 and B*39:06 are susceptible alleles in T1D, after accounting for linkage disequilibrium (LD) with HLA class II alleles, whereas other HLA-A and B alleles, including B*07:02, are protective (23). In T1D, the contribution of HLA class II to escape the elimination of autoreactive thymocytes without central tolerance and via presentation of autotopic peptides to CD4+ T cells is a key mechanism (24). However, class I, independent of class II, is also relevant (25). In general, women are more frequently affected than men, with a ratio of 3:1, indicating a hormonal involvement in the development of endocrine autoimmune diseases (3, 26). In humanized mice, HLA-DR4-dependent mechanisms regulate the sex bias of arthritis (27), and in humans, sex impacts the association of HLA-DRB1 and DQB1 with T1D (28). Thus, we hypothesized that HLA association with PGA is sex related and that differences may be noted between the two AITD phenotypes.

**Materials and Methods**

**Patients**

A total of 637 white subjects, i.e., 158 unrelated, consecutive patients with adult type 3 PGA, followed at the academic tertiary referral Orphan Center for Autoimmune Endocrine Diseases of the Johannes Gutenberg University Medical Center (Mainz, Germany), and 479 unrelated, healthy controls were included. The study was approved by the Ethics Committee of the Medical Chamber of the State of Rhineland-Palatinate, Germany, and was performed according to the Helsinki Declaration. Written, informed consent was obtained from each investigated individual. Clinical diagnoses of T1D and AITD were based on medical history, clinical phenotype, and endocrine and serological investigations, following standardized protocols and diagnostic criteria. HT was defined as the presence of at least a fivefold increase in serum antibody levels to thyroperoxidase, with or without positive thyroglobulin autoantibodies; a diagnosis of euthyroidism or hypothyroidism; a heterogeneous hypoechic pattern in thyroid ultrasound imaging; and, when available, a low uptake in the thyroid radionuclide scintigraphy (29–31). GD was defined as having positive thyroid-stimulating hormone receptor antibodies, suppressed baseline thyroid-stimulating hormone, elevated free thyroid hormones (free triiodothyronine and/or free thyroxine), and enhanced vascularization at thyroid ultrasound (“thyroid inferno”), with or without clinical manifestations of orbital disease (29–32).

A total of 354 healthy subjects from a stem cell donor registry (Westdeutsche SpenderZentrale, Ratingen, Germany; mean age 28.9 years ± 11.50, 60% women) from the same geographical region were included as sex-matched controls with respect to class I HLA-A and -B alleles. For HLA class II, DRB1, DQA1, and DQB1 alleles, 125 unrelated, healthy controls from a Johannes Gutenberg University control panel were included (43.8 ± 15.6 years, 58% women). Of these, 10 also had HLA-A and HLA-B determined as well.

**HLA typing**

All subjects were typed for HLA-A, -B, -DRB1, -DQA1, and -DQB1 alleles at a two-field level. DNA was prepared from EDTA-anticoagulated venous blood, either by an automated method (BioRobot EZ1; Qiagen, Hilden, Germany) or by a manual DNA extraction kit (QiAmp DNA Blood Mini Kit; Qiagen), according to a modified standard protocol. In a first step, HLA-A, -B, -DRB1, -DQA1, and -DQB1 alleles were amplified in a locus-specific PCR reaction, followed by hybridization with allele-specific oligonucleotide probes coupled to fluorescent beads (PCR-sequence-specific oligonucleotide; Immucor Lifecodes, Ninlen, Belgium). Data were acquired on a Luminex 200 Fluoroanalyzer with the Luminex xPONENT software v.3.1 (Luminex, Austin, TX), followed by the assignment to HLA alleles by the Immucor MATCH IT! DNA software versions 1.1–1.2.4. Samples with ambiguous typing results were further tested by high-resolution PCR-sequence-specific primers (Olerup, Vienna, Austria), following the manufacturer’s protocol and data analysis by the Helmborg Score Software (Olerup). All data were entered as two-field results.

**Statistical analyses**

The statistical analysis was exploratory. To limit inflation of type I error as much as possible, priority was given to the main
question of this investigation: is there a sex-specific association between PGA phenotype (compared with control) and the MHC class I HLA-A locus? In addition, we investigated the same question for the locus HLA-B and the MHC class II loci HLA-DRB1, -DQB1, and -DQA1. We fitted logistic models to allele-level data that contain one HLA locus, with rare alleles grouped together in one category, sex as a second factor, and the interaction between the two. The primary statistical test was a likelihood ratio test for the interaction effect. We present model parameter estimates as ORs with 95% CIs, with the most frequent allele as the reference category. In a refined analysis, we respectively compared the subgroups PGA with HT and PGA with GD to controls, and we report similar tests for interaction. For the sake of completeness, we also analyzed the association between disease and allele without regard to sex by means of the exact Fisher test, with $P$ values obtained by Monte Carlo simulations with at least 150,000 replications each. All reported $P$ values are two sided. We did not perform multiplicity adjustments to control the family-wise type 1 error. For analyses, we used SAS statistical software (SAS Institute, Cary, NC).

## Results

### Demographic data

The demographic data of the patients with adult type 3 PGA and the distribution of the endocrine components within the PGA collective are shown in Table 1.

### MHC class I HLA-A alleles

A total of 23 different HLA-A alleles were detected at a two-field level in the complete patient collective and 32 alleles within the patients and healthy controls. For statistical comparisons, all but the five most frequent alleles were considered as one group. Allelic frequencies did not significantly vary between patients and controls [Fig. 1(a)]. However, the allelic frequency distribution of the most prevalent five alleles and all others grouped together was different in male and female patients (Table 2; $P = 0.0001$), with A*02:01, *03:01, and *11:01 more frequent in male than in female patients. Consequently, the association between the five most frequent HLA-A alleles and type 3 PGA differed between male and female patients (test for interaction with sex, $P = 0.0065$; Fig. 2), both in strength and direction of the association. With the use of the most frequent allele, HLA-A*02:01, as the reference allele (Tables 2 and 3), A*11:01 was over-represented in men (OR 1.49, 95% CI 0.55 to 3.88) but under-represented in women (OR 0.42, 0.12 to 1.17), and the set of all other alleles was under-represented in men (OR 0.37, 0.18 to 0.72, $P = 0.0046$). Likewise, A*24:02 was under-represented in male patients (OR 0.37, 0.11 to 1.04) but not in women (OR 1.19, 0.65 to 2.15). With the differentiation between HT and GD, PGA patients with HT showed a similar pattern of differential allele disease association in men and women (test for interaction, $P = 0.010$; Table 4). In the HT collective, HLA-A*11:01, *24:02, and the set of all others were differentially associated with PGA in men vs women with the same pattern of directions as with PGA overall. The smaller subcollective of PGA with GD showed a similar pattern ($P = 0.24$) (33).

**A post hoc** power calculation was performed. With the use of the observed multinomial distribution of sex by HLA-A allele (a $2 \times 6$ table), we simulated diseased and control collectives with respective sample sizes of 316 and 728 (33); a total of 10,000 simulations were run. We tested for interaction between sex and HLA-A allele with the likelihood ratio test with five degrees of freedom and observed 9889 rejections, which resulted in an estimated power of 0.989 (95% CI 0.987 to 0.991).

### MHC class I HLA-B alleles

Forty-one different HLA-B alleles were determined within the patient panels, and 58 alleles were found for the patients and controls as a group. All but the most frequent six alleles were grouped together for statistical evaluation. With the consideration of all patients, HLA-B was strongly associated with PGA ($P < 0.0001$; Fig. 1(b)]. Compared with the most frequent allele B*07:02, B*08:01 (OR 7.90, 4.19 to 15.91, $P < 0.0001$), B*15:01 (OR 4.74, 2.37 to 10.0, $P < 0.0001$), and B*18:01 (OR 5.07, 2.44 to 11.00, $P < 0.0001$) were more frequent in patients; however, this was not sex related (test for interaction, $P = 0.55$). With regard to B*08:01, the ORs were 8.66, 3.73 to 23.75, and 7.24, 3.24 to 18.55, $P < 0.0001$, for HT and GD, respectively. As a consequence of the abundance of risk alleles, HLA-B*07:02 was under-represented in all patients (OR 0.42, 0.22 to 0.75, $P = 0.0666$, compared with all but the six most frequent alleles). B*39:06 was also more frequent in patients ($n = 9$) than in...
controls (n = 1); this frequency was too low for further consideration (33).

**MHC class II HLA alleles**

The most frequent nine HLA-DRB1, eight DQA1, and nine DQB1 alleles were considered for statistical evaluation, grouping the less frequent alleles into one set. All three loci were strongly associated with type 3 PGA (P < 0.0001). DRB1*03:01, *04:01, and *04:04 were more frequent in patients with PGA than among controls (33). Furthermore, DQA1*01:01, 01:02, 01:03, 02:01, 03:01, 03:03, and 05:01 were more frequent in PGA patients (33). In addition, DQB1*02:01, 02:02, 03:02, 05:01, 05:02, 06:03, and 06:04 were more frequent in patients than in controls (33). Overall, no sex-related association with PGA was registered for DRB1, DQA1, and DQB1 (test for interaction, P = 0.65, 0.67, and 0.49, respectively).

**Discussion**

This translational study reports a sex-related association of MHC class I HLA-A locus and adult type 3 PGA. We have demonstrated that sex alters the HLA-A allele disease association and described which alleles might be responsible for this effect. We demonstrate that in male patients, HLA-A*02:01 and A*11:01 are associated with a higher rate of the most prevalent PGA type. In comparison, in women A*24:02 was over-represented, whereas A*11:01 was protective. Probably as a result of a limitation of sample size, the role of particular alleles could not be statistically confirmed. As these effects were similar for the HT and GD subcollectives, T1D, rather thanAITD, is the main trigger. As a result of LD, A*11:01 is frequently inherited together with DRB1*11:01. As shown previously, this allele is protective within the haplotype DRB1*11:01-DQA1*05:05-DQB1*03:01 in type 3 PGA (19). However, the latter study and another one on MHC class I and T1D that also identified A*11:01 as protective were conducted, irrespective of the patient’s

<table>
<thead>
<tr>
<th>HLA-A Allele Type n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>98 (100)</td>
</tr>
<tr>
<td>Women</td>
<td>218 (100)</td>
</tr>
</tbody>
</table>

The number of affected individuals and the percentage (in parentheses) within the respective sex are given for the five index alleles and all “Other” alleles. P = 0.0001, Fisher’s exact test based on a Monte Carlo simulation with 1,000,000 simulation runs.
As in this paper, no sex effect on the \textit{DRB1} allele association with PGA was noted, the A*11:01 effect must be independent from LD.

Sex interaction with the HLA region represented by two single-nucleotide polymorphisms has been demonstrated for systemic lupus erythematosus, with male patients having significantly higher risk allele frequencies than women (34). Sexual disparities at the HLA locus were addressed as the main impact on genetic variation in the overall risk for systemic lupus erythematosus between men and women, independent of the X-chromosome and hormonal differences. However, in our study, the sex effect was exclusively observed within the HLA locus and not over the entire HLA region, which leaves an interaction with additional factors as a possible explanation. Women, in general, are affected more frequently by autoimmune diseases as a result of hormonal influences. Estrogens and androgens affect both innate and adaptive immune systems and strongly modulate the T helper cell type 1/2 balance (3, 26). Furthermore, the gut microbiome, which impacts the innate and adaptive immunity, interacts with sex hormones to modulate disease progression and sex bias (35). Sex hormone receptors are expressed on B and T cells, monocytes, and macrophages (36) that play a key function in the adaptive immune system by presenting antigens to T cells via the specific binding of processed antigen fragments to the HLA-binding pocket of monocytes/macrophages. Thus, sex hormones may directly or indirectly influence HLA, restricted antigen presentation and the induction of the autoimmune response. In the case of an HLA-A allele association with PGA, this would prompt the presentation of intracellular peptides to CD8+ T cells to induce a cytotoxic response.

The findings of a sex-specific allele association between HLA-A and PGA has been an interesting finding of a study intended to explore a general HLA class I association with PGA. The robustness of our findings certainly relies on whether the hypothesis was pre-specified, which is not the case. For the time being, we can only report our results, which show fairly strong effects. To have sufficient power and to limit restriction as a result of multiplicity, we focused on only the five most frequent alleles and grouped the rarer ones into one category. We considered the test for interaction between allele type and sex as the primary hypothesis test. To support this primary hypothesis further, we tested for dependency between allele type and sex in the diseased population. Although the reliance on the fact that HLA-A allele frequencies are not sex related in healthy populations, this also implies that the allele-disease association is sex related. Of course, these findings have to be confirmed in a replication study.

### Table 3. Association Between MHC Class I HLA-A Alleles and Adult Type 3 PGA Stratified by Sex

<table>
<thead>
<tr>
<th>HLA Allele</th>
<th>Men</th>
<th>OR (95% CI)</th>
<th>Women</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Cases</td>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>01:01</td>
<td>34</td>
<td>16</td>
<td>1.05 (0.52–2.09)</td>
<td>81</td>
</tr>
<tr>
<td>02:01</td>
<td>94</td>
<td>42</td>
<td>1.00</td>
<td>117</td>
</tr>
<tr>
<td>03:01</td>
<td>51</td>
<td>15</td>
<td>0.66 (0.33–1.28)</td>
<td>59</td>
</tr>
<tr>
<td>11:01</td>
<td>12</td>
<td>8</td>
<td>1.49 (0.55–3.88)</td>
<td>20</td>
</tr>
<tr>
<td>24:02</td>
<td>24</td>
<td>4</td>
<td>0.37 (0.11–1.04)</td>
<td>42</td>
</tr>
<tr>
<td>Other</td>
<td>79</td>
<td>13</td>
<td>0.37 (0.18–0.72)</td>
<td>115</td>
</tr>
</tbody>
</table>

ORs and 95% CIs refer to the risk of PGA for the index alleles compared with the reference allele HLA-A*02:01. High ORs indicate an increased risk for PGA. Profiles differ between men and women; test for effect modification of HLA-A by sex: P = 0.0065, likelihood ratio test.
DRB1-DQB1 haplotypes identified that adjusted the MHC class I effect for LD with the HLA class II alleles. In comparison, both DRB1*03:01-DQA1*05:01-DQB1*02:01 and the frequent haplotype A*02:01-B*07:02 were over-represented in Iranian T1D male patients (28). In another study, DRB1*03:01/*09:01 was mainly associated with female T1D patients in contrast to DRB1*03:01/*04 in male patients (37). In our paper, DRB1*03:01, DQB1*02:01 (DR3-DQ2), DRB1*04:01, and DQB1*03:02 (DRB1-DQ8) were more frequent in all patients with PGA; however, there was not a sex effect. Differential allele frequencies in patients with varying ethnicities and genetic backgrounds and/or the contribution of the second glandular autoimmunity in our PGA patients are potential alternative explanations. Whether further factors, such as X-chromosomal inactivation effects, contribute to the observed sex impact in the HLA association remains a subject of debate.

Earlier studies have demonstrated the association of HLA class I HLA alleles with monoglandular autoimmunity or PGA without sex differentiation (21, 23, 38, 39). In our study, HLA-B*07:02 was protective for PGA, independent of HT or GD. B*07:02 is closely linked with DRB1*15:01-DQA1*01:02-DQB1*06:02, and both the alleles and haplotype were reported as protective for glandular autoimmunity (12, 40). Thus, it is either an isolated HLA-B*07:02 effect, or this can be attributed to LD with HLA class II alleles. In comparison, both HLAB*08:01 and the frequent haplotype A*01:01-B*08:01-DRB1*03:01-DQA1*05:01-DQB1*02:01 significantly enhance the risk for the type 3 PGA phenotype, especially with GD as an AITD component. As previously reported, the susceptibility alleles DRB1*03:01 and DQB1*02:01 for T1D as endocrine components of type 3 PGA seem to be related to the combination of positively charged Lys71 and Arg74 (41). Thus, the HLA-B*08:01 effect might also be an LD-driven DRB1*03:01 rather than an isolated HLA-B effect. However, both, HLA-B and HLA-A are associated with T1D, independently of DRB1 and DQB1 (25). A large study on T1D multiplex pedigrees that adjusted the MHC class I effect for LD with the DRB1-DQB1 haplotypes identified B*07:02 as an independently protective allele but did not label B*08:01 as a susceptible allele (23). Furthermore, we found a higher B*39:06 frequency in our patients, in line with previous reports, where this HLA B allele seemed to modulate the risk of special DRB1-DQA1-DQB1-linked haplotypes in T1D (23, 42). Finally, with regard to MHC class II HLA alleles, differentiation of our PGA patients, according to sex or AITD type, did not offer novel data compared with a previous report from our laboratory (19).

## Acknowledgments

The authors are grateful to Elisa Schulze and Tanja Diana for data collection and editorial assistance, as well as to Ina Kuhn, Anne Janson, and Monika Steitz for excellent technical expertise.

**Correspondence and Reprint Requests:** George J. Kahaly, MD, PhD, Molecular Thyroid Research Laboratory, Johannes Gutenberg University Medical Center, Langenbeckstrasse 1, 55131 Mainz, Germany. E-mail: george.kahaly@unimedizin-mainz.de.

**Disclosure Summary:** The authors have nothing to disclose.

## References


