Association of HLA-DQB1*0301 and HLA-DQB1*0602 with Different Subtypes of Gastric Cancer in Taiwan

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Gastric cancer (GC) is a heterogeneous disorder with multifactorial etiologies. Genetic predisposition, environmental factors, and *Helicobacter pylori* infection are thought to interact in the manifestation of GC. Particular human leukocyte antigen (HLA) alleles play a pivotal role in cellular immunity and may be an important genetically determined host trait. To elucidate the association between the genotype of HLA class II genes and the clinical phenotype of GC, polymorphisms of HLA-DRB1 and HLA-DQB1 were determined by polymerase chain reaction with sequence-specific primers in 106 Taiwanese patients with GC and in 208 healthy controls. Comparison of allele frequencies between GC patients and healthy controls showed no significant difference at the HLA-DRB1 locus. Patients with GC had a higher frequency of DQB1*0602 (9.4% vs. 3.6%, \(P=0.05\), odds ratio 2.79, 95% confidence interval 1.41–5.47) and a lower frequency of DQB1*0301 (14.6% vs. 23.8%, \(P=0.05\), odds ratio 0.55, 95% confidence interval 0.35–0.85) compared to healthy controls. Correlation of HLA-DQB1 status with clinicopathologic features revealed predominance of male gender (16/3 vs. 50/37, \(P<0.05\)) and proximal location (12/7 vs. 28/59, \(P<0.05\)) in patients with positive HLA-DQB1*0301 compared to those with negative HLA-DQB1*0602. In contrast, a higher ratio of diffuse/intestinal subtype (20/10 vs. 30/46, \(P=0.05\)) and a lower seropositivity of *Helicobacter pylori* (14/30 vs. 58/76, \(P=0.005\)) were noted in patients with positive HLA-DQB1*0301 compared to those with negative HLA-DQB1*0301. In conclusion, HLA-DQB1*0301 confers susceptibility to gastric cancer, especially for male Taiwanese and proximal tumor location, while HLA-DQB1*0602 may have a protective effect on GC, probably through resistance to *Helicobacter pylori* infection. HLA-DQB1 alleles are associated with susceptibility or resistance to GC and also influence its clinical features.

Key words: Gastric cancer — *Helicobacter pylori* — HLA — Host susceptibility — Molecular epidemiology

Gastric cancer (GC) remains a common disease throughout the world.1) Despite some improvement in its treatment, the 5-year survival rate of GC remains low. Therefore, further exploration of etiologies and biological features of GC is mandatory to improve the diagnosis and management. Both environmental and genetic factors are crucial in the multistage model of gastric tumorigenesis. GC exhibits marked intestinal subtype in histology and anatomic involvement. Histologically, GC can be subdivided into intestinal and diffuse subtypes according to the presence of different precancerous lesions and glandular formation.2) Anatomically, GC can be divided into tumors involving the cardia (proximal) and noncardia (distal). A striking difference in clinicopathologic characteristics and epidemiologic trends was noted between different subtypes of GC.3, 4) As a result, recent investigations have focused on whether different etiologies, host susceptibility, and thresholds to exposure of cancer-causing agents may play a role in such heterogeneity.5–8)

In the sequential changes from gastritis to GC, the major environmental risks identified are dietary factors and *Helicobacter pylori* (*H. pylori*) infection.1, 9) Abundant epidemiologic data have documented that *H. pylori* is closely related not only to GC, but also to peptic ulcer disease and gastric lymphoma.10) It is worth noting that *H. pylori* infection increases the risk of both histologic subtypes of GC, but decreases the risk only in cardia cancer.11) Furthermore, the majority of *H. pylori*-infected patients develop asymptomatic gastritis and only a small portion of them may progress to GC.12) The variable outcome of *H. pylori* infection was thought to be dependent on the extent and severity of gastric inflammation.13) Accordingly, factors involved in initiation and regulation of the inflammatory response may confer host susceptibility to or against *H. pylori* infection.

Inflammatory responses to infectious agents are influenced by interindividul genetic variations.14) Genes of the
human leukocyte antigen (HLA) family are excellent candidates for examination of such genetic variation because their extensive polymorphism may play a pivotal role in controlling inflammatory and immune reactions.\(^\text{15}\) HLA class II loci encode cell surface heterodimers that are crucial in presenting peptide antigens to T-cells. A number of studies have revealed that different alleles at the HLA class II loci contribute to different susceptibility or resistance to \textit{H. pylori} infection.\(^\text{16–20}\) Moreover, the presence of particular alleles, DQB1*0301 and DRB1*1601, has been reported to be associated with increased risk of GC in Caucasian patients.\(^\text{20, 21}\) Nevertheless, conflicting results exist for HLA association with GC,\(^\text{22–23}\) possibly because of differences in ethnic background and methodology, as well as disease heterogeneity.\(^\text{24}\) To further elucidate the role of HLA in gastric carcinogenesis, we investigated the association between HLA polymorphism and GC with respect to \textit{H. pylori} infection and clinical phenotype in the Taiwanese population.

**MATERIALS AND METHODS**

**Study subjects** This was a hospital-based case-control study conducted at the National Taiwan University Hospital between 1998 and 1999. Cases who received gastrectomy for GC were prospectively enrolled. Pathological diagnosis was confirmed by the same pathologist (Chia-Tung Shun) using formalin-fixed and hematoxylin and eosin-stained tissue sections. A total of 117 eligible cases were identified and 106 (90.6\%) were successfully genotyped. These tumors were classified into 56 intestinal and 50 diffuse type, and into 19 early and 87 advanced GC. Thirty percent of cases with GC were carriers of specific alleles. Correction for multiple comparisons was made, taking into account the number of alleles studied. A \textit{P} value less than 0.05 was considered statistically significant.

**Statistical analysis** Allele frequencies were compared using a \(2 \times 2\) contingency table and the chi-square test. When appropriate, Fisher’s exact test was used, depending on the sample size of a given test. Odds ratios and 95\% confidence interval were calculated for the disease in the carriers of specific alleles. Correction for multiple comparisons was made, taking into account the number of alleles studied. A \textit{P} value less than 0.05 was considered statistically significant.

**RESULTS**

The DNA typing of HLA-DRB1 and HLA-DQB1 polymorphism is summarized in Tables II and III. The analysis of DRB1 allele frequencies (Table II) showed no significant difference between control individuals and patients with GC. The frequency of the DQB1*0301 allele was significantly reduced in patients with GC when compared with controls (14.6\% vs. 23.8\%, \textit{P}<0.05; odds ratio 0.55, 95\% confidence interval 0.35–0.85) (Table III). Moreover,
Table I. Nucleotide Sequences and Specificities of the PCR Primer Pairs Used for Identification of DRB1 (1–16) and DQB1 (17–31) Alleles

| 5′-primer sequence | 3′-primer sequence | Size of PCR product (bp) | Amplified specificities |
|--------------------|--------------------|--------------------------|------------------------|
| 1 5′-TTGTGGCACGCTTAAGTTTGAA-3′ | 5′-CTGCACGTGTAAGCTCCTCA-3′ | 255 | DRB1*01 |
| 2 5′-TTCTGTGTCGCTTAAAGG-3′ | 5′-CCACCGCGCCGCGCCGCG-3′ | 208 | DRB1*15 |
| 3 5′-TTCTCTGTGCCAGCTAAGAGG-3′ | 5′-AGGTGTCGCCGCGCGCG-3′ | 214, 220 | DRB1*16 |
| 4 5′-GACGGACGGTGTGCGGTA-3′ | 5′-GCTCCGTCACCGCCCGGA-3′ | 98 | DRB1*0301, 0304, 0305 |
| 5 5′-TACTTCTCAACCAGGAGGAGG-3′ | 5′-TCTGCACTAGTGTGCCACCC-3′ | 153 | DRB1*0301, 0302, 0303, 0305 |
| 6 5′-GTTCCTTGAGGACGAGTTTAAACA-3′ | 5′-CGCACCGACTCCCTTTGGTG-3′ | 100 | DRB1*04 |
| 7 5′-CCTGTGACGGTAAAGTTAA-3′ | 5′-CCCGTAGTTTGTCGACAC-3′ | 232 | DRB1*07 |
| 8 5′-AGTACTCTCAAGGCTAAGTGG-3′ | 5′-TGCTAGTAGGTTGACCGAC-3′ | 162, 214 | DRB1*08 |
| 9 5′-GACGGAGGGGTTGCGCTG-3′ | 5′-CCCGTAGTTTGTCGACAC-3′ | 193 | DRB1*09 |
| 10 5′-CGGTTGCTGAAACAGGGC-3′ | 5′-TGACTGAGTACCTGACCC-3′ | 198 | DRB1*10 |
| 11 5′-GTTTCTTGGGAGTACTCTGTC-3′ | 5′-TGCTGGCTGTTCAGTACTCTC-3′ | 167, 177 | DRB1*11 |
| 12 5′-CCATAAACCAGGAGGGAGCC-3′ | 5′-CTGACTGTAAGCTCTTTCCA-3′ | 184 | DRB1*12 |
| 13 5′-CCTTCTCTGAGTACTCTCGTC-3′ | 5′-CCCGCTCGTCTCTCCAGGA-3′ | 202 | DRB1*1102, 1114, 1116, 1118–21, 1301–04, 1306, 1308–10, 1312, 1315, 1316, 1319, 1322 |
| 14 5′-CCTTTTTGAGTACTCTACGC-3′ | 5′-CCCGCTCGTCTCTCCAGGA-3′ | 201 | DRB1*1101, 1103–06, 1109–12, 1115, 1122, 1305, 1307, 1311, 1314, 1318, 1321 |
| | 5′-AGTACTCTACGGTTGAGTTGTT-3′ | 201, 211 | DRB1*1401, 1404, 1405, 1407, 1408, 1411, 1414, 1418 |
| 15 5′-CGTCTGATGTGGAGGAGCATC-3′ | 5′-CGCGCTCGTCTTTCCAGG-3′ | 170 | DRB1*1401, 1407, 1410 |
| 16 5′-GCTTTGGGAGACGGTTAAAAACA-3′ | 5′-CTGTGTCAGTGGCTCGCA-3′ | 1446 | DRB1*1401 |
| 17 5′-ACCGAGGGCGGTTGCGGCGG-3′ | 5′-GCTGTTCATCTCAGGCAA-3′ | 128 | DQB1*0501 |
| 18 5′-GTTCCGCTTGTCAAGCGAC-3′ | 5′-GCTGTTCATCTCAGGCAA-3′ | 117 | DQB1*0502 |
| 19 5′-GTCGGGCTTTGACCCGAC-3′ | 5′-GGCGGGTACCCGCCGA-3′ | 87 | DQB1*0503 |
| 20 5′-GGACGGTAGGCTGCGGTTA-3′ | 5′-GCA(A)GATCCCGCGG(A)ACG-3′ | 215 | DQB1*0601 |
| | (G) | (T) | |
| 21 5′-CGTGCCGTTTGTGACCCAGTA-3′ | 5′-CAACCTCGGCCGGGCCGCCTCC-3′ | 157 | DQB1*0602 |
| 22 5′-GGACGGCGGCGGGCTGCTG-3′ | 5′-GCTGTTCATCTCAGGCA-3′ | 127 | DQB1*0603, 0607 |
| 23 5′-TCGTCGCCATTTAAGGCTCA-3′ | 5′-GCA(G)GATCCCGCCG(T)ACC-3′ | 254 | DQB1*0604/0609 |
| | (A) | (A) | |
| 24 5′-CGTGGCCTTTGAGCTACCATG-3′ | 5′-GCA(G)GATCCCGGC(GT)ACC-3′ | 206 | DQB1*0605/0609 |
| 25 5′-GTCGGCTTCTGAGTCGACAGA-3′ | 5′-TGCAAGGTCGCGGGAGCT-3′ | 205 | DQB1*02 |
| 26 5′-GACCAGGGCGGCGGCTGCTC-3′ | 5′-CTGGTGCACTACTCCGGGC-3′ | 129 | DQB1*02, 0302 |
| 27 5′-GGACGGAGGGCGTCGGTTGTA-3′ | 5′-CAGTACTGCGGCTGCTCCG-3′ | 122 | DQB1*0301, 0304 |
| 28 5′-GACGGAGGCGGCGTGGCTCTC-3′ | 5′-CAGTACTGCGGCTGCTCCG-3′ | 122 | DQB1*0302, 0303 |
| 29 5′-GCCGCTGGGGCGCCCTGTA-3′ | 5′-TGCAAGGTCGCGGGAGCT-3′ | 123 | DQB1*0301, 0303 |
| 30 5′-TCACCAACGGGACCGGACTG-3′ | 5′-TGCTAGGTTGTGCTGCACACG-3′ | 200 | DQB1*0401 |
| 31 5′-CAGGACGGCGGCGGCTGCTC-3′ | 5′-TGCTAGGTTGTGCTGCACACG-3′ | 200 | DQB1*0402 |
the DQB1∗0602 allele was more frequently found in patients with GC than controls (9.4% vs. 3.6%, *P*<0.05; odds ratio 2.79, 95% confidence interval 1.41–5.47). The haplotype analysis revealed that the frequency of DRB1∗15-DQB1∗0602 was higher in patients than in controls (40/212 vs. 15/416, *P*<0.05). The frequencies of haplotypes DRB1∗11-DQB1∗0301 and DRB1∗12-DQB1∗0301 were lower in patients than in controls (11/212 vs. 35/416, and 20/212 vs. 59/416, *P*<0.05 respectively). We further analyzed whether there was any association between these two specific DQB1 alleles and clinical features of GC. No significant association was found between these HLA alleles and the age of the patient, tumor staging, or lymph node metastasis of GC (Table IV). Correlation of HLA-DQB1 status with clinicopathologic characteristics revealed that GC with HLA-DQB1∗0602 was associated with male gender (16/3 vs. 50/37, *P*<0.05) and proximal location (cardia and body) (12/7 vs. 28/59, *P*<0.05). In contrast, GC with HLA-DQB1∗0301 tended to have a higher ratio of diffuse/intestinal subtype (20/10 vs. 30/46, *P*<0.05) and a lower seropositivity of *H. pylori* (14/30 vs. 58/76, *P*<0.005).

DISCUSSION

Although environmental factors play a major role in the development of GC, various host factors may also influence patients’ susceptibility to GC, even following the same exposure to these environmental factors. Indeed, studies of familial clustering, racial differences in incidence, and twins have all demonstrated that genetic factors play an important role in GC susceptibility.29) Specifically, recent studies have documented that HLA typing may help identify those individuals at risk.25) Herein we report for the first time such an analysis of HLA-DRB1 and DQB1 polymorphisms in Taiwanese patients with GC. Our results provide further evidence that HLA class II genes are indeed important determinants of disease susceptibility and behavior in GC, a notion previously proposed by Rigas.24) The most striking finding is that DQB1∗0301 and DQB1∗0602 play different roles in the development of GC; DQB1∗0301 may be protective, while DQB1∗0602 may be predispositional. Furthermore, for the first time we demonstrated that these two genotypes may influence tumor subtypes and gender in patients with GC.

Several other studies have been conducted to investigate the relative risk of HLA class II alleles in GC in different ethnic groups.20–23) In Caucasians, Lee et al.21) and Magnusson et al.20) have reported that HLA-DQB1∗0301 and -DRB1∗1601 are the principal susceptibility alleles for GC. Ohmori et al. have shown a slightly different frequency of DQB1∗0503 between GC patients and controls among Japanese.23) The above studies were performed by DNA genotyping of HLA.20–21, 23) However, a study from South Africa by serotyping reported the lack of such an association between GC and HLA in Cape Coloureds.22) We speculate that these conflicting results may be in part caused by differences in methodology of HLA typing and ethnic background. The genotype status of HLA may vary among different ethnic populations. Other explanations may include intrinsic heterogeneity in GC tumorigenesis and phenotype, which can be further subclassified according to the anatomical location and the histological change.

The recognition of divergent clinical, epidemiological, and genetic features in different subtypes of GC supports the idea that GC is a heterogeneous disorder.3–8) Therefore, studies employing a limited number of cases will afford inconclusive findings. To circumvent these drawbacks, we

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**Table II. Frequencies of the DRB1 Alleles in Taiwanese Gastric Cancer Patients and Controls**

| DRB1 alleles | Controls (n=208) | Gastric cancer (n=106) |
|-------------|-----------------|-----------------------|
| 01          | 2 (0.5%)        | 2 (0.9%)              |
| 04          | 50 (12.0%)      | 31 (14.6%)            |
| 07          | 12 (2.9%)       | 6 (2.8%)              |
| 08          | 50 (12.0%)      | 24 (11.3%)            |
| 09          | 62 (14.9%)      | 36 (17.0%)            |
| 10          | 8 (1.9%)        | 3 (1.4%)              |
| 11          | 38 (9.1%)       | 12 (5.7%)             |
| 12          | 64 (15.4%)      | 22 (10.4%)            |
| 13          | 15 (3.6%)       | 8 (3.8%)              |
| 14          | 20 (4.8%)       | 15 (7.1%)             |
| 15          | 41 (9.9%)       | 27 (12.7%)            |
| 16          | 22 (5.3%)       | 8 (3.8%)              |
| 17          | 32 (7.7%)       | 18 (8.5%)             |

* P=0.0018 by chi-square test, *P*=0.023 after Bonferroni correction.

**Table III. Frequencies of the DQB1 Alleles in Taiwanese Gastric Cancer Patients and Controls**

| DQB1 alleles | Controls (n=208) | Gastric cancer (n=106) |
|-------------|-----------------|-----------------------|
| 02          | 44 (10.6%)      | 23 (10.8%)            |
| 0301        | 99 (23.8%)      | 31 (14.6%)            |
| 0302        | 31 (7.5%)       | 22 (10.4%)            |
| 0303        | 62 (14.9%)      | 37 (17.5%)            |
| 0401        | 20 (4.8%)       | 11 (5.2%)             |
| 0402        | 4 (1.0%)        | 3 (1.4%)              |
| 0501        | 13 (3.1%)       | 8 (3.8%)              |
| 0502        | 41 (9.9%)       | 20 (9.4%)             |
| 0503        | 11 (2.6%)       | 6 (2.8%)              |
| 0601        | 61 (14.7%)      | 24 (11.3%)            |
| 0602        | 15 (3.6%)       | 20 (9.4%)             |
| 0603        | 3 (0.7%)        | 0 (0%)                |
| 0605        | 12 (2.9%)       | 7 (3.3%)              |

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enrolled a number of patients with GC and a matched control group in a rather homogeneous ethnic population. In this situation, we noted that HLA-DQB1*0602 was frequently associated with GC with proximal location, while HLA-DQB1*0301 confers protection mainly for GC of the diffuse subtype. Collectively, our findings support the notion that HLA genotyping helps identify the clinical subtype of GC and susceptibility to GC. They also imply that stratification of GC into different subtypes is mandatory for meaningful molecular and genetic studies in the future.7)

Male preponderance is a well-known clinical characteristic of GC. Most studies, including those from Taiwan, have documented that the male/female ratio in GC is approximately two.26, 30) The cause of this male preponderance is not known, and may be due to a combination of genetic and environmental factors. It is noteworthy that GC with positive HLA-DQB1*0602 was predominantly associated with the male gender (male/female ratio, 5.33), indicating that this allele plays a more important role in male patients. We do not believe that such male predominance appeared by chance, because the study patients were not pre-selected and the overall male/female ratio was 1.65, which is in the range of those previously published from Taiwan.30) An association of HLA with gender is also found in diseases other than GC. In Hodgkin’s disease and multiple sclerosis,31, 32) some alleles were more frequently found in female patients, while a male-associated susceptibility genotype was seen in acute lymphoblastic leukemia.33) One possible explanation for this gender-related difference is that different humoral and cellular immune responses may be associated with different HLA alleles in different genders.34, 35) Another possibility is that sex hormones or sex-linked gene inheritance may be responsible for the enhanced susceptibility.32)

A puzzling feature of gastric carcinogenesis is that clinically evident diseases occur in only a small proportion of patients with H. pylori infection.10) One proposal is that genetically determined differences in immune responses may play a role. The genetic basis for the susceptibility to H. pylori infection is not fully understood, but HLA alleles may play a role, as proposed by a number of authors.16–20)

What has emerged from these studies is that DQB1*0401 and DQ5 are positively associated with the development of atrophic gastritis, while the lack of DQA1*0102 is associated with a higher frequency of atrophic gastritis and gastric adenocarcinoma after acquisition of H. pylori infection.16–20) In this study, we found that DQB1*0301 was associated with a lower risk of GC and a lower seropositivity of H. pylori. It remains to be determined whether the apparent association of H. pylori negativity with DQB1*0301 is merely accidental or actually reflects the fact that DQB1*0301 confers protection against GC through resistance to H. pylori infection. Our results differ

| Characteristics | HLA-DQB1*0301 | HLA-DQB1*0602 |
|-----------------|---------------|---------------|
| Gender          |               |               |
| Male            | 15            | 16            |
| Female          | 15            | 3             |
| Tumor location  |               |               |
| Proximal (cardia/body) | 10       | 20            |
| Distal (angle/antrum) | 20       | 56            |
| Tumor stage     |               |               |
| Early           | 6             | 4             |
| Advanced        | 24            | 15            |
| Lymph node metastasis |           |               |
| Positive        | 21            | 14            |
| Negative        | 9             | 5             |
| Histologic subtype |             |               |
| Diffuse         | 20            | 12            |
| Intestinal      | 10            | 7             |
| H. pylori seropositivity |       |               |
| Positive        | 14            | 13            |
| Negative        | 16            | 6             |

a) P=0.037, b) P=0.012, c) P=0.012, d) P=0.003.
infection runs a chronic or self-limited course. Further, interleukin-1 may affect whether a primary influence in the expression of tumor necrosis factor-

HLA, other factors may contribute to the variable outcomes of H. pylori infection. Besides HLA, other factors may contribute to the variable outcomes of H. pylori infection. For example, the genetic influence in the expression of tumor necrosis factor-α and interleukin-1 may affect whether a primary H. pylori infection runs a chronic or self-limited course. Further studies centering on the interplay between host immunogenetic factors and H. pylori infection may help elucidate the different outcomes of H. pylori-related diseases.

Although our findings suggest that GC subtypes and the patients’ genetics might have a strong association with HLA, the mechanism remains unknown. One likely explanation is that gender and DQB1 allele type influence the development of GC subtypes following an etiological event such as H. pylori infection. If this is the case, certain alleles may do this by modulating the presentation of infection-derived peptides to T-cells. In turn, the magnitude or type of T-cell response may influence the proliferation of a lineage-specific malignant clone. Nevertheless, one cannot rule out that HLA polymorphism may directly be linked to certain susceptibility genes. Further studies to correlate GC subtypes and different immunogenetic factors may help to clarify the development and progression of GC.

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