

# HLA Alleles and Susceptibility to Dermatological Disorders Associated with *Helicobacter pylori* Infection: a Significant Association to HLA-Cw\*06

( dermatological disorders / *Helicobacter pylori* / HLA-A, -B, -C, -DRB1, -DQB1 allele frequencies )

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**Abstract.** The occurrence rate of HLA class I and class II alleles was established in 24 patients suffering from dermatological disorders associated with the *Helicobacter pylori* infection. The increased frequency of HLA-C\*0602, 4 was found to be 0.1875 compared to 0.0733 in the control group (odds ratio: 2.913; two-sided P value: P = 0.0251). Our data suggest that the HLA-Cw6 molecule play a role in the susceptibility to the *Helicobacter pylori* infection.

*Helicobacter pylori* is a gramnegative bacillus, which is an important aetiopathological factor of chronic gastritis, gastric and duodenal ulcers, and gastric adenocarcinomas (Blaser, 1992; Bayerdorffer et al., 1995; Cover and Blaser, 1996). An association between *H. pylori* infection and chronic *urticaria*, *rosacea*, Sjögren's syndrome, and other dermatological disorders was observed; moreover, a beneficial effect of *H. pylori* eradication in their treatment was reported, too (Kolibášová et al., 1994; Rebora et al., 1994; Bohmeyer et al., 1996).

The induction of an autoimmune response is one of the mechanisms by which *H. pylori* could induce gastritis, gastric and duodenal ulcers, and perhaps also dermatological disorders (Negrini et al., 1996; Appelmelk et al., 1998). Since associations between some autoimmune diseases and HLA antigens/alleles are well established (Todd et al., 1988; Hammer et al., 1995), we have decided to look for such a possible association by investigating HLA allele frequencies in patients suffering from dermatological disorders associated with the *H. pylori* infection. According to our knowledge, no similar studies have been reported so far.

## Material and Methods

HLA-A, -B, -C, -DRB1 and -DQB1 allele frequencies were investigated in 24 patients suffering from dermatological disorders associated with *H. pylori* infection (*urticaria chronica* – 8 patients, *rosacea* – 11 patients, *eczema chronicum* – 5 patients). The urea breath test and the enzyme-linked immunosorbent assay were used for the laboratory diagnosis of *H. pylori* infection. All patients were *H. pylori* positive and they were subjected to detailed clinical examinations. The diagnosis of the *H. pylori* gastritis was confirmed by an oesophagogastroduodenoscopy in 9 patients; no patient suffered from a gastric or duodenal ulcer. The control group represented 109 unrelated healthy individuals typed for HLA-A, -B, -C alleles (Kulcsárová et al., 2000), and 143 unrelated healthy individuals typed for HLA-DRB1, DQB1 alleles (Fazekašová et al., 1998), respectively.

## DNA extraction

Genomic DNA was isolated from peripheral blood leucocytes obtained from EDTA-treated blood. The DNA was prepared by the salting-out method. Erythrocytes were destructed in red-cell lysis buffer (0.32 M saccharose, 1% Triton X-100, 5 mM MgCl<sub>2</sub>, 12 mM Tris, pH 7.5). A centrifugation for 5 min followed; the pellet was then rinsed with water and resuspended in 300 µl of nuclei lysis buffer (0.375 M NaCl, 0.12 M EDTA, pH 8.0), 10 µl of 1% SDS and 100 µl of 5 M NaCl. Five hundred µl of phenol-chloroform were added after 20 min incubation, and the mixture was vortexed and centrifuged for 10 minutes. DNA aspirated from the top aqueous phase was precipitated with 95% ethanol, washed in 70% ethanol and resuspended in distilled water.

## Amplification conditions

Dynal HLA-A, -B low-resolution-SSP (Dynal, Oslo, Norway) and Olerup SSP HLA-C low-resolution (Olerup SSP AB, Oslo, Norway) primer sets were used. They contained 5'- and 3'-primers for the identification

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of alleles A\*0101 to A\*8001, B\*0702 to B\*8201 and Cw\*0102 to Cw\*1802. Twenty four PCR reactions for HLA-A, 48 for HLA-B and 20 for HLA-Cw were performed per sample in a reaction volume of 10 µl, not including a 10 µl mineral oil overlay. Twenty four primer mixes specific to the second exon were used to identify HLA-DRB1 alleles (20 for identification of DR1-DR16, three for DR51, DR52, and one for DR53 specificities). Similarly, 8 primer mixes were used for the identification of HLA-DQ1-DQ10 alleles. Each tube in the set contained a primer solution consisting of a specific primer mix, i.e. the allele- and group-specific primers as well as a control primer pair matching non-allelic sequences. One hundred and ten ng DNA, 5 µl of a PCR-solution 10x (PCR buffer, 50 mM MgCl<sub>2</sub>, 200 µM of dNTP, glycerol, cresol red, respectively), 0.4 U Taq-polymerase (GIBCO, London, U.K.), and 5 µl of a primer mix formed the PCR reaction mixtures. PCR amplifications were carried out in a thermocycler

"PTC-100™-Programmable Comprised Thermal Controller" (MJ Research, Inc., Watertown, MA). Cycling parameters were: 2 min at 94°C, followed by 10 cycles of 10 s at 94°C, and 60 s at 65°C, 20 cycles of 10 s at 94°C, 50 s at 61 °C, and 30 s at 72°C.

### Gel electrophoresis

PCR products were electrophoresed in 1% agarose gels containing 0.5 µg/ml ethidium bromide. The gels were run for 15 min at 15 V/cm in 0.5x TBE buffer and visualized using UV illumination.

The Fisher's exact test was used to test significance of allele frequency differences.

### Results

The occurrence rates of HLA-A, -B, -C alleles in the investigated group of patients are given in Table 1. The most frequent HLA-A alleles were A\*0201-22 (0.2333) and A\*0301-3 (0.2000); the most common HLA-B alle-

Table 1. Frequencies of HLA-A, -B and -C alleles in *H. pylori*-positive patients and healthy controls

| HLA-A    | f controls<br>N=218 | f patients<br>N=48 | HLA-B    | f controls<br>N=218 | f patients<br>N=48 | HLA-C           | f controls<br>N=218 | f patients<br>N=48 |
|----------|---------------------|--------------------|----------|---------------------|--------------------|-----------------|---------------------|--------------------|
| A0101-2  | 0.1284              | 0.1333             | B0702-8  | 0.0872              | 0.1333             | Cw0101-3        | 0.0229              | 0.0333             |
| A0201-22 | 0.2615              | 0.2166             | B0801-4  | 0.0826              | 0.0333             | Cw02021-3       | 0.0412              | 0.0167             |
| A0301-3  | 0.1376              | 0.2000             | B1301-4  | 0.0596              | 0.0667             | Cw0302-9        | 0.0733              | 0.0833             |
| A2301    | 0.0275              | 0.0667             | B1401-2  | 0.0183              | 0.0333             | Cw0401-6        | 0.1559              | 0.1333             |
| A2402-14 | 0.0917              | 0.1167             | B1501-37 | 0.0505              | 0.0500             | Cw0501-2        | 0.0412              | 0.0167             |
| A2501-2  | 0.0367              | 0.0667             | B1801-5  | 0.0779              | 0.1167             | <b>Cw0602,4</b> | <b>0.0733</b>       | <b>0.1875</b>      |
| A2601-8  | 0.0550              | 0.0167             | B2701-11 | 0.0596              | 0.0333             | Cw0701-10       | 0.2293              | 0.3167             |
| A1101-4  | 0.0596              | 0.0500             | B3501-21 | 0.1009              | 0.0833             | Cw0801-4        | 0.0321              | 0.0667             |
| A2901-3  | 0.0138              | 0.0000             | B3701-2  | 0.0046              | 0.0500             | Cw1202-6        | 0.1468              | 0.1167             |
| A3001-4  | 0.0183              | 0.0167             | B3801-2  | 0.0550              | 0.0333             | Cw1301          | 0.0000              | 0.0000             |
| A31012   | 0.0138              | 0.0000             | B3901-12 | 0.0137              | 0.0500             | Cw1402-3        | 0.0000              | 0.0000             |
| A3201-2  | 0.0321              | 0.0333             | B4001-10 | 0.0321              | 0.0500             | Cw1502-6        | 0.0137              | 0.0000             |
| A3301-3  | 0.0046              | 0.0167             | B4101-2  | 0.0137              | 0.0000             | Cw1601-4        | 0.0183              | 0.0000             |
| A3401-2  | 0.0092              | 0.0000             | B4201-2  | 0.0046              | 0.0000             | Cw1701-2        | 0.0229              | 0.0000             |
| A3601    | 0.0092              | 0.0000             | B4402-10 | 0.0917              | 0.0667             | Cw1801-2        | 0.0091              | 0.0000             |
| A4301    | 0.0000              | 0.0000             | B4501    | 0.0046              | 0.0000             | Cx              | 0.1192              | 0.0000             |
| A6601-3  | 0.0092              | 0.0000             | B4601    | 0.0046              | 0.0000             |                 |                     |                    |
| A6801-3  | 0.0138              | 0.0167             | B4701-2  | 0.0091              | 0.0167             |                 |                     |                    |
| A6901    | 0.0046              | 0.0333             | B4801-3  | 0.0000              | 0.0000             |                 |                     |                    |
| A7401-3  | 0.0092              | 0.0167             | B4901    | 0.0137              | 0.0333             |                 |                     |                    |
| A8001    | 0.0000              | 0.0000             | B5001-2  | 0.0046              | 0.0167             |                 |                     |                    |
| Ax       | 0.0642              | 0.0000             | B5101-9  | 0.0412              | 0.0167             |                 |                     |                    |
|          |                     |                    | B5201    | 0.0091              | 0.0167             |                 |                     |                    |
|          |                     |                    | B5301-2  | 0.0046              | 0.0333             |                 |                     |                    |
|          |                     |                    | B5401    | 0.0046              | 0.0000             |                 |                     |                    |
|          |                     |                    | B5505    | 0.0137              | 0.0167             |                 |                     |                    |
|          |                     |                    | B5601-3  | 0.0183              | 0.0000             |                 |                     |                    |
|          |                     |                    | B5701-4  | 0.0275              | 0.0167             |                 |                     |                    |
|          |                     |                    | B5801-2  | 0.0137              | 0.0167             |                 |                     |                    |
|          |                     |                    | B5901    | 0.0000              | 0.0000             |                 |                     |                    |
|          |                     |                    | B6701    | 0.0045              | 0.0000             |                 |                     |                    |
|          |                     |                    | B7301    | 0.0000              | 0.0000             |                 |                     |                    |
|          |                     |                    | B7801-2  | 0.0046              | 0.0167             |                 |                     |                    |
|          |                     |                    | B8101    | 0.0000              | 0.0000             |                 |                     |                    |
|          |                     |                    | B8201    | 0.0046              | 0.0000             |                 |                     |                    |
|          |                     |                    | Bx       | 0.0642              | 0.0000             |                 |                     |                    |
| Total    | 1.0000              | 1.0000             |          | 0.9992              | 1.0000             |                 | 0.9991              | 1.0000             |