HLA-DQ Alleles in White and African American Patients With Juvenile-Onset Recurrent Respiratory Papillomatosis

Lucie Gregoire, PhD; Patrick M. Reidy, MD; Raja Rabah, MD; Wayne D. Lancaster, PhD

Objective: To determine HLA-DQ*050X and -DQB1 allele associations in juvenile-onset recurrent respiratory papillomatosis (RRP) for risk, disease course, and human papillomavirus type.

Design: A nonrandomized controlled study was performed on DNA extracted from papilloma specimens of children with a history of RRP, and from peripheral blood of African American and white children without RRP. The frequencies of DQ*050X and DQB1 alleles were compared between patients and ethnically matched controls.

Subjects: Records of 48 children treated for RRP at Children’s Hospital of Michigan in Detroit (26 African American and 22 white) were reviewed. Control subjects consisted of 80 African American and 80 white children seen at the hospital for conditions other than RRP.

Results: African American and white patients with DQB1*050X (not *0501, *0502, *0503, *0504, or *0505) were at higher risk to develop RRP than controls (P = .01 and .03, respectively). DQB1*0502 was protective for African Americans (P = .01). Whites with DQA1*0102 were at risk for RRP (P = .03). This allele was associated with disease remission in African Americans (P = .03). DQ*0101/0104 conferred protection in whites (P = .047).

No association was seen for allele frequency and human papillomavirus type. Whites with haplotype DQ*0501/DQB1*0201 were at high risk for RRP (P = .002). No relationships were seen for African Americans or whites between haplotype frequencies and disease course or human papillomavirus type.

Conclusions: HLA-DQ*050X and -DQB1 alleles occur at different frequencies in African American and white children with RRP than controls. Specific alleles influence risk for RRP. Allele and haplotype frequencies have some influence on disease course, but were independent of human papillomavirus type.

Arch Otolaryngol Head Neck Surg. 2003;129:1221-1224

UVEENILE-ONSET recurrent respiratory papillomatosis (RRP) is a serious, chronic illness caused by human papillomavirus (HPV). According to a report from the National Registry for Juvenile-Onset Respiratory Papillomatosis, the lesions are most commonly found in the larynx and can manifest themselves anywhere from the age of 1 month to 16 years, although a peak in the average age at diagnosis appears at year 2. The HP types 6 and 11 are the most frequently detected genotypes of the more than 100 HPV genotypes known to date. The clinical course of the disease varies from patient to patient. Some patients have few recurrences and experience spontaneous remission, while other patients have frequent recurrences with the need for repeated surgical excisions to relieve their symptoms. A small number of patients (approximately 1%) develop a disseminated form of the disease, which can progress to laryngeal or bronchogenic carcinoma.

Our group has previously reported that patients with lesions containing HPV-11 have a more aggressive course than patients with HPV-6–associated lesions. These patients were diagnosed at a younger age, had longer periods of disease activity, required more surgical procedures, and were less likely to go into remission than HPV-6–infected patients. In addition, we found that there was a higher incidence of HPV-11 infection in African American than white patients (odds ratio, 7.0; 95% confidence interval, 0.04-0.45; P = .008). The factors accounting for the differences in disease course and virus prevalence are unknown. It is thought, however, that genetic polymorphisms underlying the function of the immune system may play a role in the variable nature of RRP.

The HLA system is a large gene region on the short arm of chromosome 6.
It contains more than 100 genes, most of which code for proteins involved in regulating the function of the immune system. One of these functions is to defend the body against foreign pathogens, such as viruses. Viral proteins are processed into small peptide fragments, which are then displayed on the surface of cells bound to HLA molecules. HLA molecules with bound peptides are recognized by T lymphocytes (especially virus-specific cytotoxic CD8+ T cells) and an immune response against the foreign peptides is generated. Previous research has shown that virus-specific humoral, natural killer cell, and macrophage-mediated immunity is generally intact in patients infected with HPV. This has led researchers to consider CD8+ T lymphocytes as the defective element underlying papilloma-induced disease.

Evidence supporting a role for the HLA system in eradicating lesions caused by HPV comes from observations that certain HLA alleles are associated with risk of cervical intraepithelial neoplasia and cervical cancer. Class II HLA alleles have also been assessed in RRP. One study, in which 16 cases of RRP were analyzed, showed enrichment for the DQ3 locus. This work is difficult to interpret, however, because of the small number of cases. Aaltonen et al were unable to show a difference in DQx or DQB1 allele frequencies between patients with adult-onset RRP and the reference population. They did not find an association between HLA-DQx alleles and HPV type or number of surgical procedures. This may be a reflection of the poor immune response these patients have to HPV. The present study was conducted to analyze HLA-DQx and -DQB1 allele frequencies in African American and white children with juvenile-onset RRP and to determine whether there are associations between allele frequencies and risk of RRP, disease course, and HPV type.

### Methods

#### Patients

Records from 48 patients diagnosed as having RRP at the Children’s Hospital of Michigan, Detroit, whose DNA from papilloma specimens could be analyzed were reviewed as previously reported. Demographic data were collected regarding the current disease status of each patient. The patients were categorized as currently having active disease or as being in remission, which was defined as the absence of laryngeal symptoms for 1 year with or without negative results of laryngoscopy. Detection and typing of HPV were performed by means of the polymerase chain reaction on DNA extracted from paraffin-embedded samples of each patient’s papillomas. For this study, reference samples were obtained by collection of peripheral blood from 80 African American and 80 white children without a history of RRP. This study was approved by the Human Investigation Committee of Wayne State University.

### DNA Extraction

The DNA was extracted from formalin-fixed, paraffin-embedded RRP as previously described. The DNA was extracted from heparinized peripheral blood by means of a kit (Instagene Whole Blood Kit; Bio-Rad Laboratories, Hercules, Calif) according to the manufacturer’s protocol.

### HLA-DQx AND -DQB1 Typing

HLA-DQx and -DQB1 alleles were determined by polymerase chain reaction and single-strand oligonucleotide probe hybridization. DQx alleles were determined according to Kimura et al. DQx alleles were amplified by means of DQxP1 (5’-ATGGTGTAACTTGTACCAGT-3’) at the 5’ end and DQxP2 (5’-TGGTGAGCAGCGTGATTG-3’) at the 3’ end to generate a 230-base pair fragment. Fourteen single-strand oligonucleotide probes discriminated between HLA-DQx*0101/0104, *0102, *0103, *0201, *0301/0302, *0401, *0501, and *0601. DQB1 alleles were determined as previously described, and the single-strand oligonucleotide probes could discriminate between HLA-DQB1 *0201/0202, *0301, *0302, *0303, *0304, *0401, *0402, *0501, *0502, *0503, *0504, *0505, *0601, *0602, *0603, *0604, and *0605. Amplification products whose hybridization pattern failed to coincide with the pattern of a known allele were classified as unknown alleles. Exceptions were the unknown alleles of the DQB1*03, *05, and *06 families, which were designated *030X, *050X, and *060X, respectively.

The African American and white groups were analyzed independently with regard to the frequency of HLA-DQx alleles, DQx alleles, and DQx-DQB1 haplotypes. Allele and haplotype frequency in each group was then analyzed with respect to the type of HPV (6 or 11) present in each patient’s RRP, as well as with regard to the current status of disease activity in each patient.

### Statistical Analysis

HLA-DQx and -DQB1 allele frequencies were compared by Fisher exact test (2-sided). Means were analyzed by the unpaired t test (2-tailed with Welch correction). P < .05 was considered significant.

### Results

#### HLA-DQx Alleles

HLA-DQx allele frequencies are summarized in Table 1. Analysis of HLA-DQx alleles showed a higher risk for white patients to develop RRP if they expressed the DQx*0102 allele (P = .03). Paradoxically, the DQx*0102 allele was associated with disease remission.
in African American patients. The frequency of this allele in 17 patients with active disease was 11.8% (4/34), while in 9 patients in remission it was 38.9% (7/18) (odds ratio, 0.21; 95% confidence interval, 0.05-0.85; P = .03). There was no statistically significant difference in the number of years from onset of disease between these 2 groups of African American patients (active, 5.8 ± 3.6 years; remission, 4.0 ± 3.2 years). Of the 7 of 10 white patients with DQ*0102 whose data could be evaluated for disease status, none of the 8 white patients with active disease had DQ*0102 and only 7 of 28 with the allele were in remission (P = .31). DQ*0101/0104 was found to be a protective allele in white patients (P = .047) but had no association in African American patients. There was no association between any of the HLA-DQα alleles and the presence of HPV-6 or -11 DNA.

**HLA-DQB1 ALLELES**

HLA-DQB1 allele frequencies are summarized in Table 2. Their analysis showed that both African American and white patients with DQB1*050X (not *0501-5) were at higher risk to develop RRP compared with patients with other alleles (P = .01 and .03, respectively). The DQB1*0402 allele was found to be protective in African Americans (P = .01), but had no association in whites. There was no association between the presence of HLA-DQB1 alleles and HPV type, nor did allele frequencies correlate with active disease or remission.

**HLA-DQα/DQB1 HAPLOTYPES**

The African American and white patients compared with regard to the frequency of HLA-DQα/DQB1 haplotypes. Of the most common DQα/DQB1 haplotypes for these 2 ethnic groups, only DQα*0501/DQB1*0201 showed an association for risk of RRP in the white patients (P = .02; odds ratio, 9.59; 95% confidence interval, 2.32-39.68).

**COMMENT**

HLA-DQα proteins are expressed on antigen-presenting cells and are responsible for presenting foreign particles, such as viral antigens, to T lymphocytes. Unlike other proteins in the HLA system, HLA-DQ molecules are highly polymorphic. The structure of the HLA-DQ peptide–binding groove varies considerably depending on which of the DQα and DQB1 alleles are being expressed.13 These differences may affect the immune response generated by the HLA system by increasing or decreasing the ability of HLA-DQ molecules to bind and properly present foreign antigens to T lymphocytes.14

The relationship between the HLA system and an increased susceptibility to viral infection has been known since the late 1960s, when polymorphisms in HLA-DQ molecules were shown to be associated with a higher risk of developing Hodgkin disease, a lymphoma associated with Epstein-Barr virus.15 Previous studies have also shown an association between the expression of certain HLA-DQ alleles and cervical neoplasia, a disease caused by the HPV. Not only are specific alleles associated with cervical neoplasia, but also the frequency of HLA-DQ alleles differs in ethnically distinct populations.

Our results demonstrate an association between HLA-DQα and -DQB1 alleles and HPV-induced juvenile-onset RRP. Moreover, we found that the frequency of these alleles was different for the African American and white populations. The HLA-DQα*0102 allele conferred a statistically significant risk to white patients for the development of RRP, but was associated with disease remission in African American patients. This seemingly paradoxical finding in African American patients, with DQα*0102 showing no association with risk for developing RRP but showing an association with remission, is likely due to the fact that RRP develops on the background of a naïve immune system, whereas remission is due to cell-mediated immunity. The DQα*0101/0104 allele was found significantly more often in the white reference population, suggesting a protective effect against the development of RRP.

Similarly, high-risk and protective alleles were found for HLA-DQB1. The presence of DQB1*050X conferred an increased risk to both African American and white patients for the development of RRP; this was statistically significant (P = .01 and .03, respectively). This finding may be misleading, however, since our probes could not distinguish whether 1 or more alleles are present in this group. African American patients appeared to be protected if they expressed the DQB1*0402 allele (P = .01).

**Table 2. HLA-DQB1 Allele Frequencies in Patients With Laryngeal Papilloma and Local Control Subjects**

<table>
<thead>
<tr>
<th>HLA-DQB1 Allele*</th>
<th>White, No. (%)†</th>
<th>African American, No. (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP</td>
<td>LC</td>
</tr>
<tr>
<td>*0201/0202</td>
<td>11</td>
<td>25.0</td>
</tr>
<tr>
<td>*0301</td>
<td>9</td>
<td>20.5</td>
</tr>
<tr>
<td>*0302</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td>*0303</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td>*0305</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>*030X</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td>*0402</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>*0501</td>
<td>5</td>
<td>11.4</td>
</tr>
<tr>
<td>*0502</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>*0503</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>*050X</td>
<td>3</td>
<td>6.8</td>
</tr>
<tr>
<td>*0601</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>*0602</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>*0603/0607</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td>*0604/0608</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td>*0605/0609</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>*060X</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Unknown</td>
<td>5</td>
<td>11.4</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>100</td>
</tr>
</tbody>
</table>

Abbreviations: LC, local control; LP, laryngeal papilloma.
*Alleles *0304, *0401, *0504, *0505, and *0606 also were assayed but were not represented in either patient or control samples.†Because of rounding, percentages may not total 100.
*P = .01; odds ratio, 0.67; 95% confidence interval, 0.004-1.22.
†P = .03; odds ratio, 17.46; 95% confidence interval, 0.88-345.68.
‡P = .01; odds ratio, 4.62; 95% confidence interval, 1.44-14.87.
The African American and white populations in our study were also examined with regard to the frequency of HLA-DQα/DQB1 haplotypes. Only one of the common haplotypes found in white patients (DQα*0501/DQB1*0201) showed increased risk for RRP. Aside from comparing the frequency of HLA-DQ alleles between African American and white patients with RRP and their ethnically matched reference populations, the expression of specific DQα and DQB1 alleles was also compared with the type of HPV present in each patient. No association was found between HPV type and either DQα or DQB1 alleles. This result was similar to that found by Aaltonen et al.8

Finally, after a medical chart review was performed, patients were classified either as having active or recurrent disease or as being in remission. The current status of each patient’s disease was then compared with the presence of HLA-DQα and -DQB1 alleles. HLA-DQα*0102 was the only allele found to have a statistically significant association with disease status. In African American patients, this allele was associated with disease remission (P = .03). It was not associated with disease status in white patients.

Since it is believed that papilloma regression is a function of the cell-mediated immune response, it is not surprising to see little association between class II HLA-DQ allele frequencies and the course of RRP. HLA-DQ associations with respect to risk of RRP are consistent with a class II (humoral) response. The lack of association between virus type and allele frequency could be a reflection of the high degree of homology between HPV-6 and HPV-11 and thus similar epitopes recognized by the immune system.

It should be recognized that association studies as described here are prone to type I errors. False-positive results can occur because alleles do not necessarily act independently and there may be an unrecognized bias in patient or local control selection. Additional studies will be needed to resolve this issue.

CONCLUSIONS

Our research has demonstrated that HLA-DQα and -DQB1 alleles are found in different frequencies in African American and white children with RRP compared with ethnically matched reference populations. Specific alleles increase or decrease the risk of RRP and may influence the course and prognosis of the disease. Our study did not show a relationship between the presence of specific HLA-DQ alleles and type 6 or 11 HPV.

Submitted for publication August 9, 2002; final revision received February 25, 2003; accepted February 28, 2003.

Corresponding author and reprints: Lucie Gregoire, PhD, Department of Immunology and Microbiology, Wayne State University School of Medicine, 540 E Canfield Ave, Detroit, MI 48201 (e-mail: gregoire@genetics.wayne.edu).

REFERENCES