Association of HLA-DR4 (DRB1*0404) With Human Papillomavirus Infection in Patients With Focal Epithelial Hyperplasia

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Objectives: To determine gene frequencies of HLA-DR alleles in 22 Mexican patients with focal epithelial hyperplasia and compare them with those present in ethnically matched healthy subjects, as well as to determine the types of human papillomavirus present in the lesions.

Design: Prospective and retrospective observational study.

Setting: Dermatology outpatient clinic in a general hospital.

Patients: Twenty-two patients with clinically and histologically confirmed focal epithelial hyperplasia seen within a 10-year period.

Interventions: None.

Main Outcome Measures: Results of high-resolution DNA typing for HLA-DR alleles and biopsy for viral typing.

Results: HLA-DR4 (DRB1*0404) was significantly increased (P<.001; odds ratio, 3.9; 95% confidence interval, 1.86-8.03). Seventeen (85%) of 20 patients had human papillomavirus subtype 13. The data on human papillomavirus differed from reports elsewhere that described association with human papillomavirus type 32.

Conclusions: The HLA-DRB1*0404 allele suggests that Amerindian populations are at risk, and in this group, the Mexican population studied was affected only by human papillomavirus type 13.

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Focal epithelial hyperplasia (FEH) is an uncommon proliferative disease of the oral mucosa, caused by the human papillomavirus (HPV) types 13 and 32. It presents clinically as multiple pink and smooth-surfaced papular formations 1 to 5 mm in diameter with a tendency to coalesce. These lesions are usually asymptomatic, but can on occasion be quite painful and of cosmetic concern. (Figure 1). Focal epithelial hyperplasia predominantly affects children, but its true prevalence is unknown because most reports are based on isolated case reports, and only a few include large number of patients. In Mexico, a previous study by our group found a prevalence of 0.026% in the past 10 years at a dermatology outpatient clinic.

Focal epithelial hyperplasia is a peculiar infection with lesions persisting for many years. There is no specific treatment for this disease, although there have been successful results with carbon dioxide laser, surgical excision, cryosurgery, and topical agents such as podophyllum resin. The factors that determine susceptibility to FEH and its transmission are still unknown, but a tendency to affect certain ethnic groups and the presence of more than 1 case of FEH in the same family suggest that genetic predisposition may be involved in the pathogenesis of this disease, and therefore the major histocompatibility complex genes are relevant to study.

The aims of this study were to determine gene frequencies of HLA-DR alleles in 22 Mexican patients with FEH and compare them with those present in ethnically matched healthy subjects, as well as to determine the types of HPV present in the lesions.

METHODS

This study included all patients with a clinical and histologically confirmed diagnosis of FEH admitted to the Dermatology Outpatient Clinic of the Hospital General “Dr Manuel Gea González,” Tlalpan, Mexico, from January 1, 1990, to December 31, 2000. This study was approved by the ethics and research committee of the hospital.

All identified patients with FEH received a questionnaire to complete demographic, epidemiologic, and clinical data, which included...
age, sex, location of the lesions, evolution time, treatment, history of another lesion associated with HPV, and existence of relatives with FEH. With previous informed consent, a peripheral blood sample (4.5 mL) was taken for DNA isolation and a 4-mm punch biopsy of a representative lesion was obtained to search for HPV in fresh or paraffin-embedded tissue by the polymerase chain reaction (PCR) method.

Blood samples were obtained from 99 healthy, unrelated Mexican mestizo individuals ethnically matched to the patients with FEH. The Mexican mestizo individuals included in the present study had 56% Native American Indian genes, 40% white genes, and 4% black genes.23

HLA-DR TYPING
Genomic DNA from whole blood containing EDTA was extracted by the salting-out technique.24 Generic HLA-DRB1 typing was performed by PCR sequence-specific oligonucleotyped reverse dot-blot hybridization (AmpliSor; Hoffmann La Roche, Basel, Switzerland). High-resolution HLA typing was performed by dot-blot hybridization of amplified DNA with sequence-specific oligonucleotide probes labeled with digoxigenin dideoxyuridine triphosphate. Information about the DRB1 sequence was obtained from the 12th International Histocompatibility Workshop.25

IDENTIFICATION OF HPV
The presence of HPV was confirmed with the use of the PCR–reverse fragment length polymorphism method. Briefly, the DNA was extracted from the tissue sections by standard techniques. The genomes of the HPV types are each unique, yet they share interspersed regions of DNA sequence homology, particularly within the open reading frame L1. We used L1 consensus primers MY11 for the 5’ region and MY09 for the 3’ region. These primers promote an amplification product of approximately 450 base pairs. We used, as an internal control, a modified plasmid that contains the identical primer binding sites as the HPV DNA target, and the product has 1200 base pairs.

The PCR products were analyzed by means of gel electrophoresis in 1.5% agarose in 0.5× TBE [Tris(hydroxymethyl) aminomethane–boric acid–EDTA] with ethidium bromide staining. The aliquots of the amplified DNA of the positive samples were digested in 2 separate reactions, one with restriction enzyme Rsal and the other with Rsal plus PstI. The digestion protein analysis was unique for each virus type.

STATISTICAL ANALYSIS
The epidemiologic data were analyzed with descriptive statistics. Nonparametric tests were used for statistical analysis of the HLA-DR alleles and the HPV type. Gene frequencies were compared between patients and controls. Their significance was analyzed with the χ² test and Fisher exact test.

RESULTS
Twenty-two patients were included in this study, 9 females (41%) and 13 males (59%). The average age at the time of diagnosis was 19 years (range, 5–49 years). Most patients (16 [73%]) were in the first 2 decades of life, and all of them were from a low socioeconomic background.

Clinical features included multiple soft papular formations with a bright and smooth surface similar in color to adjacent mucosa (Figure 1). The most common location was lower lip mucosa (n=20), followed by the upper lip (n=18), tongue (n=16), buccal mucosa (n=15), and labial commissures (n=14); 3 cases were in the soft palate. In all cases, diagnosis was histologically confirmed (Figure 2).

Eleven patients had previously received treatment to their lesions, which consisted of surgical excision, an-
Gene Frequencies of HLA-DR Alleles and HLA-DRB1*04 Subtypes in Patients With FEH and Healthy Controls

<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>No.</th>
<th>Gene Frequency</th>
<th>No.</th>
<th>Gene Frequency</th>
<th>P Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR4</td>
<td>24</td>
<td>0.545</td>
<td>47</td>
<td>0.237</td>
<td>&lt;.001</td>
<td>3.86 (1.86-8.03)</td>
</tr>
<tr>
<td>DR8</td>
<td>7</td>
<td>0.159</td>
<td>33</td>
<td>0.165</td>
<td>.92</td>
<td>NA</td>
</tr>
<tr>
<td>DR14</td>
<td>7</td>
<td>0.159</td>
<td>21</td>
<td>0.105</td>
<td>.46</td>
<td>NA</td>
</tr>
<tr>
<td>DR10</td>
<td>2</td>
<td>0.045</td>
<td>1</td>
<td>0.005</td>
<td>.15</td>
<td>NA</td>
</tr>
<tr>
<td>DR11</td>
<td>2</td>
<td>0.045</td>
<td>20</td>
<td>0.100</td>
<td>.38</td>
<td>NA</td>
</tr>
<tr>
<td>DR11</td>
<td>1</td>
<td>0.022</td>
<td>10</td>
<td>0.050</td>
<td>.69</td>
<td>NA</td>
</tr>
<tr>
<td>DR16</td>
<td>1</td>
<td>0.022</td>
<td>5</td>
<td>0.025</td>
<td>.66</td>
<td>NA</td>
</tr>
</tbody>
</table>

HLA-DRB1*04 Subtypes

<table>
<thead>
<tr>
<th>Allele</th>
<th>No.</th>
<th>Gene Frequency</th>
<th>No.</th>
<th>Gene Frequency</th>
<th>P Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*0404</td>
<td>8</td>
<td>0.181</td>
<td>9</td>
<td>0.045</td>
<td>.004</td>
<td>4.67 (1.50-14.34)</td>
</tr>
<tr>
<td>*0407</td>
<td>6</td>
<td>0.136</td>
<td>21</td>
<td>0.106</td>
<td>.75</td>
<td>NA</td>
</tr>
<tr>
<td>*0411</td>
<td>4</td>
<td>0.090</td>
<td>3</td>
<td>0.015</td>
<td>.03</td>
<td>6.5 (1.17-38.37)</td>
</tr>
<tr>
<td>*0410</td>
<td>2</td>
<td>0.045</td>
<td>2</td>
<td>0.010</td>
<td>.31</td>
<td>NA</td>
</tr>
<tr>
<td>*0403</td>
<td>2</td>
<td>0.045</td>
<td>4</td>
<td>0.020</td>
<td>.66</td>
<td>NA</td>
</tr>
<tr>
<td>*0408</td>
<td>2</td>
<td>0.045</td>
<td>1</td>
<td>0.005</td>
<td>.15</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; FEH, focal epithelial hyperplasia; NA, not applicable; OR, odds ratio.

tiseptic mouthwashes, antifungal agents, vitamins, BCG vaccination, elastic collodion with salicylic acid, and cryosurgery; however, all of them failed to control the disease. Duration of the lesions ranged from 1 month to 34 years, with a mean of 8.3 years. Sixteen (73%) of 22 patients mentioned a family history of FEH.

Association with other viral diseases was observed in 9 patients (41%); these included common warts on the dorsal surface of the hand (n=7), flat warts on the face (n=2), and viral condylomas of the penis (n=2).

Viral typing was possible in 20 cases, 17 (85%) of which were positive for HPV-13, and 3 samples were negative (15%). We did not find any patient to be positive for the HPV-32 subtype.

Nineteen (86%) of 22 patients were HLA-DR4 positive; 7 of them (37%) were homozygous. Gene frequencies of HLA-DR alleles showed a statistically significantly increased frequency of HLA-DR4 in patients with FEH when compared with ethnically matched healthy controls (P<.001; odds ratio, 3.9; 95% confidence interval, 1.86-8.03) (Table). Subtyping in HLA-DR4-positive individuals (Table) showed that HLA-DRB1*0404 was significantly increased in patients with FEH as compared with healthy controls (P=.004; odds ratio, 4.7; 95% confidence interval, 1.50-14.34).

**COMMENT**

In this study we found a significant association between FEH and HLA-DR4 (DRB1*0404). This allele is relatively frequent in American autochthonous populations, such as Mazatecans and Nahuas, as well as in the Mexican mestizo population. This finding might also explain the relatively high prevalence of FEH in other Latin American countries, especially Guatemala, where there is a low genetic admixture and HLA-DRB1*0404 is also relatively frequent.

Furthermore, we also found that 85% of patients tested for HPV were positive for HPV-13, confirming previous studies in Mexicans. Other viral diseases were either present or mentioned in 9 patients (41%), among which common warts predominated. Association of FEH with other types of HPV infections has not been well established; however, there is 1 report stating that 8 (33%) of 24 patients had other viral diseases in addition to FEH (6 with common warts and 2 with condylomas). This is an infrequently reported finding; it suggests to us that this possible association has not been intentionally sought by others workers, and therefore the actual prevalence of additional HPV infections in patients with FEH remains to be established. On the other hand, these data support a possible genetic susceptibility to infection by other HPV types in patients with FEH.

With regard to age at onset, most cases (73%) were diagnosed during the first 2 decades of life, which is similar to other studies from Guatemala and Colombia, where FEH is also particularly prevalent in young individuals, and differs from data from Eskimos and European patients, who manifest the disease usually at an older age.

The time course of the disease is difficult to establish, since the lesions are usually asymptomatic; however, in this study, evolution time ranged from 1 month to 34 years. In other reports, evolution time usually ranged from a few months, or even weeks, to a few years.

Family history of FEH was also found in 16 patients (73%), and this finding has also been mentioned in other Latin American populations. The mechanism of the association between HLA-DRB1*0404 and FEH is probably related to the infection by HPV-13, and it must be a very complex phenomenon.

Several viral peptides might be presented in major histocompatibility complex class I molecules to CD8+ cytotoxic lymphocytes, but, in addition, HPV infection in patients with FEH might generate viral peptides that need to be eliminated via class II major histocompatibility complex molecules. The latter mechanism in DRB1*0404-positive patients may be deficient, probably because of
molecular mimicry between the aforementioned viral peptides and the DRB1*0404 molecule. Alternatively, the antigen-binding region in the DRB1*0404 molecule might be deficient in anchoring viral peptides from HPV-13 infection (Figure 3). Nevertheless, further studies are needed to better understand the relationship between structure and function of the class II major histocompatibility complex molecules in the presentation of HPV-13 infection.

In summary, we propose a hypothesis that the HLA-DR locus has a role in the pathophysiology of FEH by selecting genetically susceptible individuals, particularly those homozygous for HLA-DR4 (DRB1*0404 subtypes), who have certain amino acid sequences in the third hypervariable region. This amino acid sequence in turn restricts the antigen presentation of peptides derived from the viral capsid to CD4 lymphocytes and, consequently, produces accumulation of these peptides inside the cell, thus generating the image of so-called “mitosoid” figures. HPV indicates human papillomavirus; RER, rough endoplasmic reticulum.

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REFERENCES