Further Evidence for an Association of HLA-DR7 With Basal Cell Carcinoma on the Tropical Island of Saba

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Objective: To establish the association of HLA alleles (ie, HLA-DR1, HLA-DR4, and HLA-DR7) with individuals with skin cancer on the tropical island of Saba. This island was chosen because most of the white population has fair skin and excessive exposure to sunlight, which results in a high prevalence of skin cancer.

Design: HLA typing was performed in 124 white individuals with histologically proven basal cell and/or squamous cell carcinoma and in control subjects. Skin type, the presence of freckling, and the number of actinic keratoses were determined.

Setting: Population-based study.

Subjects: Inhabitants of Saba with and without skin cancer.

Main Outcome Measures: Presence of HLA-DR1, HLA-DR4, and HLA-DR7 alleles.

Results: Associations of HLA alleles with basal cell and squamous cell carcinoma have been reported. The presence of the HLA-DR7 allele was positively associated with the development of basal cell carcinoma (odds ratio, 3.8; 95% confidence interval, 1.1-13.4). Adjustment for skin type, which is a potentially confounding factor for the association between HLA alleles and skin cancer, did not substantially alter this association. No other associations between HLA alleles and skin cancer were found, possibly because of the small size of the study population.

Conclusion: This study presented further evidence for an association between HLA-DR7 and basal cell carcinoma.

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The development of skin cancer is governed by a complex interplay of environmental and genetic factors. The most important environmental factors are exposure to sunlight, exposure to chemicals, and infection with human papillomaviruses. Genetic factors, such as skin type, freckling, tendency to develop actinic keratoses, and resistance to infection with human papillomaviruses, are also important. In addition, age and sex should always be considered.

The HLA system plays a role in the development of skin cancer, although the exact mechanisms have not yet been resolved. Associations of HLA alleles with basal cell carcinomas and squamous cell carcinomas have been described both in the immunocompetent population and in renal transplant recipients.

In the immunocompetent population, the most consistent reported association is of the HLA-DR1 allele with (usually multiple) basal cell carcinoma and a negative association of basal cell carcinoma with the HLA-DR4 allele, although these associations could not always be confirmed. Several studies of renal transplant recipients reported a positive association between the HLA-DR7 allele and squamous cell carcinomas or basal cell carcinomas.

Because of the small sample size in this study of inhabitants on the tropical island of Saba, we limited our study to the association of basal cell carcinoma and squamous cell carcinoma with the HLA alleles, which have previously been reported to be associated with one of these skin cancers (ie, HLA-DR1, HLA-DR4, and HLA-DR7).

RESULTS

RELATIONSHIP OF RISK FACTORS TO SKIN CANCER

The basic characteristics of the 124 individuals whose HLA alleles were studied are presented in Table 1. Of the 14 patients...
PATIENTS AND METHODS

SELECTION OF PATIENTS AND CASE DEFINITION

The study was performed on Saba, a small island in the Caribbean Sea. Most whites living on this island are descendants of English, Scottish, Irish, and Dutch settlers who arrived on the island between 1660 and 1685. The population born on Saba consists of about 450 whites and 500 non-whites. The white population largely consists of 5 families. Excessive exposure to sunlight and the fair skin of most of the white population result in a high prevalence of actinic keratoses and skin cancer.

The surveys were undertaken in May and November 1993. During the first visit to the island, the available data in the government registry office were used to make a list of all 256 white individuals (51% women) who were born on Saba before 1960. The list was primarily based on the family names of the 5 largest families and, with the help of government employees, was completed by adding the names of white individuals with different names who were also born on the island.

Data regarding the history of skin cancer were collected from the medical charts of all white individuals. Only histologically confirmed squamous cell carcinomas and basal cell carcinomas were counted as skin cancers.

A skin cancer clinic was announced, which resulted in 50 persons spontaneously visiting our clinic in May, 16 of whom had had skin cancer. During the second survey, an additional 87 persons were seen. Family members of patients with skin cancer from the first survey were telephoned to attend the clinic. Individuals who came to the clinic spontaneously were also included in the study.

CLINICAL INVESTIGATION

The skin of all individuals visiting our clinic was examined for the presence of skin cancer. Biopsy of the suspicious lesions was performed for histological diagnosis. Skin type was determined and actinic keratoses were counted. Freckles were recorded as present or absent. Lifetime sun exposure was assessed by questionnaire, and blood was drawn for HLA typing. Briefly, each patient’s cumulative exposure to sunlight was calculated by adding the exposure because of occupational activities (minimum 1 and maximum 8 hours per day) for different age groups to the exposure because of nonoccupational activities, such as outdoor recreation (minimum 1 and maximum 16 hours per week) and vacation. The complete questionnaire and the protocol for assessing exposure to sunlight are available on request from Dr Bouwes Bavinck.

Of the 137 persons attending the clinic, 10 refused to have blood drawn. Of the remaining 127 persons, HLA typing was performed successfully in 124 samples, which resulted in the 124 subjects analyzed in this study. One hundred nineteen individuals did not attend the clinic because they were not on the island when we were organizing the clinic, they were too busy to attend the clinic, or they did not want to participate in the study.

HLA TYPING

Typing for HLA-A, HLA-B, and HLA-DR antigens was performed with sets of well-defined alloantisera by means of the standard complement-dependent lymphocytotoxicity test for HLA-A and HLA-B, specified by the National Institutes of Health, Bethesda, Md, and the 2-color fluorescence technique for HLA-DR typing.

STATISTICS

The continuous data concerning age, sun exposure, and number of actinic keratoses did not show a normal distribution, and therefore differences between the groups of patients with and without skin cancer regarding these values were calculated using the nonparametric Wilcoxon signed rank test. Differences regarding ordinal data, such as sex, presence of freckles, skin type, and presence of certain HLA phenotypes, were calculated using χ² analysis. The HLA-DR1, HLA-DR4, and HLA-DR7 alleles were tested a priori and, therefore, correction for the number of antigens tested was not performed.

Relative risks were estimated with the exposure odds ratios by logistic regression. Considering the association between HLA alleles and skin cancer, only skin type is a potential confounder, a factor that can be adjusted for in the logistic model. Age and sex are not confounders, because although these features are risk factors for skin cancer, they do not depend on HLA genotype. Actinic keratoses are intermediate in the etiologic pathway between HLA alleles and skin cancer and therefore should not be adjusted for. Similarly, freckles should not be used in the model, since these spots can be considered an indicator for skin type.

The calculations were performed with the statistical software package JMP (version 2; SAS Institute Inc, Cary, NC) with the help of Epi Info (version 6.04b, January 1997, Centers for Disease Control and Prevention, Atlanta, Ga, and World Health Organization, Geneva, Switzerland).

POSITIVE ASSOCIATION BETWEEN HLA-DR7 AND SKIN CANCER

The basic characteristics of the HLA-DR7–positive and –negative individuals are presented in Table 2. The presence of the HLA-DR7 allele was significantly associated with a history of basal cell carcinomas only (Table 1 and Table 3). The nonadjusted odds ratio for developing basal cell carcinoma in HLA-DR7–positive individuals compared with HLA-DR7–negative individuals was 3.8 (95% confidence interval, 1.1-13.4); after adjusting for skin type, the odds ratio became 3.4 (95% confidence interval,
The odds ratios were similar in the largest 2 families (families 1 and 4), informative in the smaller families (families 3 and 5), and not informative in family 2 (Table 3). The HLA-DR7 allele was not associated with a history of squamous cell carcinoma (Table 1).

The HLA-DR1 allele was not associated with an increased risk of basal cell carcinoma; the risk for developing squamous cell carcinomas was marginally increased, but was not statistically significant (Table 1). The frequency of the HLA-DR4 allele was only slightly decreased in individuals with both types of skin cancer, and no statistically significant associations were observed between skin cancer and the other HLA-DR alleles or with homozygosity for HLA-DR (data not shown). In addition, no statistically significant association between the presence of any of the HLA-A or HLA-B alleles and any type of skin cancer was found.

**COMMENT**

A statistically significant association was found between the presence of the HLA-DR7 allele and basal cell carcinoma only, in concurrence with earlier findings in the renal transplant population. This association was independent of skin type. The direction of the association was similar in 4 of the 5 families studied, so that genetic re-

### Table 1. Baseline Characteristics of the 124 Individuals in the HLA Study*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SCC (n = 14)</th>
<th>BCC Only (n = 19)</th>
<th>No Carcinoma (n = 91)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F, No. (%)</td>
<td>7 (50)/7 (50)</td>
<td>8 (42)/11 (58)</td>
<td>41 (45)/50 (55)</td>
<td>SCC: .73</td>
</tr>
<tr>
<td>Age, mean ± SD (range), y</td>
<td>66 ± 16 (38.7-90.9)</td>
<td>64 ± 14 (35.9-82.7)</td>
<td>54 ± 13 (33.9-84.3)</td>
<td>SCC: .01</td>
</tr>
<tr>
<td>Skin type, I/II/III/IV, No.</td>
<td>6/5/3/0</td>
<td>7/8/4/0</td>
<td>24/37/24/6</td>
<td>SCC: .40</td>
</tr>
<tr>
<td>Sun exposure, mean ± SD (range), h (&lt;1000)</td>
<td>56 ± 42 (22.9-149.2)</td>
<td>49 ± 34 (7.3-133.8)</td>
<td>38 ± 27 (5.7-113.9)</td>
<td>SCC: .05</td>
</tr>
<tr>
<td>Freckles, yes/no, No. (%)</td>
<td>11 (79)/3 (21)</td>
<td>15 (79)/4 (21)</td>
<td>52 (57)/39 (43)</td>
<td>SCC: .11</td>
</tr>
<tr>
<td>No. of actinic keratoses, mean ± SD (range)</td>
<td>167 ± 94 (37-344)</td>
<td>166 ± 68 (2-308)</td>
<td>44 ± 48 (0-223)</td>
<td>SCC: &lt;.001</td>
</tr>
<tr>
<td>HLA-DR1, yes/no, No. (%)</td>
<td>7 (43)/17 (57)</td>
<td>6 (31)/14 (69)</td>
<td>19 (21)/71 (79)</td>
<td>SCC: .30</td>
</tr>
<tr>
<td>HLA-DR4, yes/no, No. (%)</td>
<td>4 (29)/10 (71)</td>
<td>5 (26)/14 (74)</td>
<td>31 (34)/79 (66)</td>
<td>SCC: .68</td>
</tr>
<tr>
<td>HLA-DR7, yes/no, No. (%)</td>
<td>1 (7)/13 (93)</td>
<td>7 (37)/12 (63)</td>
<td>12 (13)/79 (87)</td>
<td>SCC: .50</td>
</tr>
</tbody>
</table>

* SCC indicates squamous cell carcinoma; BCC, basal cell carcinoma.
† The P values of the ordinal data were calculated by the χ² test, and those of the integer data were calculated by the nonparametric Wilcoxon signed rank test.

### Table 2. Baseline Characteristics of the 124 Individuals Who Were HLA-DR7 Positive and Negative*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HLA-DR7 Positive (n = 20)</th>
<th>HLA-DR7 Negative (n = 104)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F, No. (%)</td>
<td>6 (30)/14 (70)</td>
<td>50 (48)/54 (52)</td>
<td>.13</td>
</tr>
<tr>
<td>Age, mean ± SD (range), y</td>
<td>59 ± 16 (33.9-82.7)</td>
<td>57 ± 13 (34.1-90.9)</td>
<td>.52</td>
</tr>
<tr>
<td>Skin type, I/II/III/IV, No.</td>
<td>6/11/3/0</td>
<td>31/39/28/6</td>
<td>.22</td>
</tr>
<tr>
<td>Sun exposure, mean ± SD (range), h (&gt;1000)</td>
<td>36 ± 36 (5.7-143.1)</td>
<td>43 ± 30 (63.3-149.2)</td>
<td>.11</td>
</tr>
<tr>
<td>Freckles, yes/no, No. (%)</td>
<td>9 (45)/11 (55)</td>
<td>69 (66)/35 (34)</td>
<td>.08</td>
</tr>
<tr>
<td>No. of actinic keratoses, mean ± SD (range)</td>
<td>57 ± 95</td>
<td>61 ± 61</td>
<td>.18</td>
</tr>
<tr>
<td>Skin cancer, No. (%)</td>
<td>1 (5)</td>
<td>13 (13)</td>
<td>SCC: .05‡</td>
</tr>
<tr>
<td>SCC</td>
<td>7 (35)</td>
<td>12 (12)</td>
<td>BCC: .02‡</td>
</tr>
<tr>
<td>No carcinoma</td>
<td>12 (60)</td>
<td>79 (76)</td>
<td></td>
</tr>
</tbody>
</table>

* SCC indicates squamous cell carcinoma; BCC, basal cell carcinoma.
† The P values of the ordinal data were calculated by the χ² test, and those of the integer data were calculated by the nonparametric Wilcoxon signed rank test.
‡ Versus no carcinoma.

### Table 3. Frequency of HLA-DR7 Allele in Individuals With and Without Basal Cell Carcinoma (BCC), Stratified According to Skin Type and Family*

<table>
<thead>
<tr>
<th>HLA-DR7 Allele</th>
<th>BCC Only, % Positive (Pos/Neg)</th>
<th>No Skin Cancer, % Positive (Pos/Neg)</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>37 (7/12)</td>
<td>13 (12/79)</td>
<td>3.8 (1.1-13.4)</td>
</tr>
<tr>
<td>Skin Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>43 (3/4)</td>
<td>8 (2/22)</td>
<td>8.3 (0.74-113)</td>
</tr>
<tr>
<td>II</td>
<td>25 (2/6)</td>
<td>24 (9/28)</td>
<td>1.0 (0.12-7.5)</td>
</tr>
<tr>
<td>III/IV</td>
<td>50 (2/2)</td>
<td>3 (1/29)</td>
<td>29.0 (1.2-1512)</td>
</tr>
<tr>
<td>Family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>36 (5/9)</td>
<td>15 (8/44)</td>
<td>3.1 (0.67-14.0)</td>
</tr>
<tr>
<td>2</td>
<td>0 (0/1)</td>
<td>0 (0/9)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>100 (3/0)</td>
<td>75 (6/2)</td>
<td>∞</td>
</tr>
<tr>
<td>4</td>
<td>40 (4/6)</td>
<td>20 (10/40)</td>
<td>2.7 (0.51-13.9)</td>
</tr>
<tr>
<td>5</td>
<td>100 (1/0)</td>
<td>25 (1/3)</td>
<td>∞</td>
</tr>
</tbody>
</table>

* CI indicates confidence interval; Pos, positive; Neg, negative.
† Odds ratio, adjusted for skin type, was 3.4 (and 95% CI, 1.1-13.8).
‡ Because some individuals have ancestors in more than 1 family, the total number of affected individuals may exceed the total number in the 5 families.
latedness of the individuals is not the most likely explanation for the association between the presence of the HLA-DR7 allele and basal cell carcinoma.

The positive association of HLA-DR1 with basal cell carcinoma and the negative association of HLA-DR4 with this type of tumor could not be confirmed in the small population of Saba, which is in agreement with a Swedish study.\(^{11}\) We cannot exclude, however, the possibility that an association between these alleles and skin cancer still exists but could not be detected in our study because of the small size of the study population.

This study supports the hypothesis that HLA alleles may be involved in the development of skin cancer, but no conclusions can be drawn as to the stage of tumor development in which these antigens may be important. The immune response to premalignant cells may be of equal or more importance than the response against the tumor cells. The association with HLA class II antigens indicates that during the induction phase of this immune response, CD4-positive regulatory T cells may be involved in recognizing HLA class II–associated peptides, which may be tumor antigens, viral antigens, photoantigens, or other, as yet undetermined antigens.\(^{26}\)

In conclusion, much larger studies are needed to unravel the complex associations between HLA alleles and the development of skin cancer. Data on basal cell carcinoma and squamous cell carcinoma should be analyzed separately, since the pathogenesis of and the antigenic determinants in these tumors are likely to be different.

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