Controversial data have been reported about HLA alleles and susceptibility to melanoma. The relationship between distribution of HLA alleles in patients with melanoma and susceptibility to tumour was analysed, to study the possible correlation between HLA class II DQA1, DQB1 and DRB1 genes and melanoma in a Spanish population. Genomic DNA from 82 patients with melanoma and 367 random healthy donors, from the same geographic area, were typed by PCR-SSP (sequence specific primers). The patients were also divided into different groups according to the age and presence of cancer relatives, and compared with the controls. None of these HLA class II alleles showed significant positive or negative associations with either the overall population of patients with melanoma or the considered subgroups. Moreover, values for relative risk of DQB1*0301, DQB1*0302, DQB1*0303, DQB*05, DQA1*0401, DQA1*0101/0104 and DRB*08, which have been reported to be increased or decreased in patients with melanoma, were very low and of no statistical significance. Our results indicate that HLA class II alleles may not contribute to a strong susceptibility to melanoma in the Spanish population, although further studies on larger series are needed to corroborate this. **Key words:** HLA; melanoma; susceptibility.
Genomic DNA of each of the 82 patients with melanoma was extracted using the proteinase K digestion method (14).

Control HLA class II genotype and allele frequencies were obtained from a historical database of the Centro de Transfusión de la Comunidad Valenciana comprising routine typing of solid organ and bone marrow transplant donors – 367 control individuals typed for the HLA DQA1 and DRB1 loci and 100 typed for the HLA DQB1 locus.

Genomic DNA from patients and controls were all typed by a low-resolution polymerase chain reaction (PCR) using the sequence-specific primers (PCR-SSP) method for DQB1 and DRB1, and a high-resolution PCR-SSP method for DQA1 according to previously described methods (15–16).

The significance of the association of HLA alleles with Spanish patients with melanoma was assessed by \( \chi^2 \) analysis. Fisher’s exact \( p \)-values were calculated for analyses in which one or more variables within 2 \( \times \) 2 tables were less than 5. The Bonferroni method was used to correct for the number of comparisons (P.), due to multiple comparisons, as has been recommended (17). A level of \( p < 0.05 \) was accepted as statistically significant. Estimation of the association between HLA antigens and melanoma was made using the method by Woolf for relative risk (RR, odds ratio) (18).

RESULTS

The frequencies of the HLA-DRB1, -DQA1, and -DQB1 alleles as defined by PCR-SSP in the 82 patients and in the control group, as well as in the resulting subgroups, are summarized in Tables I, II and III.

No significant differences in the frequencies of DQA1, DQB1 and DRB1 alleles between patients, subgroups and controls were found, except for a small decrease or increase in some of them.

Low-resolution analysis of DQB1 reported in Table II showed a non-significant decrease of the DQB1*0302/0307/0308 in patients with melanoma (14.63 versus 25%, \( p = 0.08; p_c = 0.56; RR = 0.51 \)).

When subgroups where considered, the DQB1*0302/0307/0308 allele was decreased in patients with melanoma with age \( \leq 30 \) years (9.4 versus 25%; \( p = 0.06; p_c = 0.42; RR = 0.42 \)), together with a non-significant increase of the DQB1*04 (10.0 versus 3%, \( p = 0.07, p_c = 0.49; RR = 3.59 \)) for patients with melanoma at age \( > 30 \) years.

Patients with a family history of cancer presented an increase of DQA1*0401 (0 versus 7.1%; \( p = 0.08; p_c = 0.96; RR = 0.88 \)). Patients with no family history showed an increase of DQA1*0103 (28.2 versus 16.9%; \( p = 0.08; p_c = 0.96; RR = 1.9 \)), DQB1*06 (51.3 versus 46%; \( p = 0.06; p_c = 0.42; RR = 1.79 \)), DQB1*04 (10.3 versus 3%; \( p = 0.07; p_c = 0.49; RR = 3.69 \)) and DRB1*0301/0304-0316 (33.3 versus 21.25%; \( p = 0.08; p_c = 1.2; RR = 1.85 \)) and a decrease of DQB1*05 (20.5 versus 37%; \( p = 0.06; p_c = 0.42; RR = 0.43 \)).

DISCUSSION

In this study we report for the first time a molecular analysis of HLA DRB1, DQA1 and DQB1 polymorphism in Spanish patients with melanoma. We found no significant differences in antigen frequencies between patients with melanoma and controls. Controversial data about the association between HLA class II antigens and melanoma have been reported and significant differences can be found among different populations (5–13). These differences are not unusual in HLA-disease association studies, which could be due to ethnic variations in HLA frequencies or to heterogeneity of genetic and environmental factors.

The DQB1*0301 has been found to be associated with melanoma in a different Caucasian ethnic group (8–9). This allele has also been found to be associated with the progression of the disease in these populations (9–10). A study from Texas found this allele in 56% of patients with melanoma versus 27% of controls (9). The authors also found that the presence of this allele was associated with thicker primary tumours and a higher

<table>
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<tr>
<th>Alleles</th>
<th>Melanoma cases</th>
<th>Age at diagnosis</th>
<th>Family history of cancer*</th>
<th>Controls</th>
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<td>9 (28)</td>
<td>13 (26)</td>
<td>11 (28)</td>
</tr>
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</table>

*Missing data in 4 cases.
probability to present with regional or distant metastatic disease. A further study in a Caucasian population in Southampton, UK, did not find a statistically significant increase in this allele in the melanoma population but of DQB1*0303 (19.2% of patients with melanoma vs. 5.8% of controls) (10). However, patients with melanoma carrying the DQB1*0301 allele were associated with thicker tumours and more advanced disease (44% patients in stages III/IV) (10), but this allele did not lead to an increased risk of relapse after a mean follow-up of 67 months.

Two recent studies in Italian populations showed somewhat different results from those found in English or North American Caucasians (11, 12). Some alleles were found with an increased frequency, but they did not reach statistical significance. Interestingly, Lulli et al. (12) found an increase in DQB1*0301 frequency but Lombardi et al. (11) found a decrease in patients with melanoma. On the other hand, the DQB1*0501 allele presented in a high percentage but there was no single allele related to clinical stage (11). Our results are somewhat similar to these studies. This could be due to a common ethnic origin, although differences can also be explained by the small sample size analysed.

Differences are even more remarkable in another study performed in a Japanese population (13). The authors found an increased frequency of the DQB1*0302 allele in patients with melanoma and a decreased frequency of the DQA1*0101, DQA1*0401 and DRB1*0802 alleles (13). Nevertheless, and like the Italian studies, none of these differences reached statistical significance (after correction of the p-value with the Bonferroni test). The association between the DQB1*0301 allele and the presence of lymph node metastasis is remarkable in this work, which supports the above-mentioned studies in English and North American populations.

The design of the present study does not allow us to evaluate the relationship between HLA allele frequencies and illness outcome, although all patients are currently present in a high percentage but there was no single allele related to clinical stage (11). Our results are somewhat similar to these studies. This could be due to a common ethnic origin, although differences can also be explained by the small sample size analysed.

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enrolled in a prospective study to evaluate the relationship between class II HLA alleles and disease recurrence. Such a discrepancy among all the studies, even though it could be due to ethnic differences, a bias in the population selection, or to a high heterogeneity in genetic and environmental factors (ultraviolet radiation, virus, trauma, and others), points to an absence of a strong role of class II HLA alleles in susceptibility to melanoma. We have not even seen statistical differences in different subgroups of patients with clinical characteristics that could indicate some kind of genetic predisposition. Nevertheless, small differences were observed and although they did not reach statistical significance, they could be explained by the sample size studied.

In summary, although melanoma is a very immunogenic tumour and HLA antigens have a basic role in the immune response to melanoma, our results suggest that HLA class II alleles may not contribute to a strong susceptibility to melanoma, at least not in Spanish patients. However, further studies with larger series are needed to exclude definitively any relationship between specific alleles and susceptibility of melanoma.

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