Acta Derm Venereol 2002; 82: 90–93

Taylor & Francis
healthsciences

INVESTIGATIVE REPORT

Molecular Genetic Analysis of HLA-DR and -DQ Alleles in Spanish Patients with Melanoma

E. NAGORE[1], M. D. PLANELLES[2], E. LEDESMA[1], J. M. MILLÁN[3], A. INSA[4], V. OLIVER[5], C. GUILLÉN[1] and J. M. FORTEA[5]

[1]*Department of Dermatology, Instituto Valenciano de Oncología,* [2]*Centro de Transfusión de la Comunidad Valenciana,* [3]*Department of Genetics, Hospital Universitario La Fe,* [4]*Department of Oncology, Hospital Clínico Universitario,* [5]*Department of Dermatology, Hospital General Universitario, Valencia, Spain*

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.***

(Accepted February 11, 2002.)

Acta Derm Venereol 2002; 82: 90–93.

Eduardo Nagore, C/Denia, 20-6, ES-46006 Valencia, Spain. E-mail: eduyame@meditex.es

It is speculated that susceptibility to melanoma is linked to human leucocyte antigen (HLA) class II genes (1). HLA class II-mediated antigen presentation to CD4-positive T cells contributes to the immune response to melanoma, while the cytotoxic activity of these CD4 T cells can be blocked by anti-HLA DR antibodies (2). However, tumours possess a range of proteins including viral antigens, tumour-specific transplantation antigens and other components that are not normally present in the host cells (3). It has been shown that normal melanocytes that express HLA II antigens can function as antigen-presenting cells, which can induce a suppression of immune tolerance and lead to autoimmune phenomena of the skin, such as vitiligo (4).

Association of HLA class II antigens with melanoma is still controversial. Early serological studies have identified an increase in HLA class II DR4 and DR5 phenotypes in patients with cutaneous malignant melanoma (5–6). On the other hand, analysis of familial melanoma suggests a genetic linkage to the HLA-encoding region on chromosome 6, indicating that HLA genes may promote predisposition to cutaneous malignant melanoma (7). This region seems to be defined by the transporter associated with the antigen processing (TAP2) gene upstream and DRB1 downstream, containing a relatively small fragment of chromosome 6 (8–9).

More recently, some alleles have been found to be increased or decreased in patients with melanoma, although there are significant differences among different ethnic groups (10–13).

The aim of this study was therefore to examine for the first time the distribution of HLA class II DQA, DQB and DRB alleles in Spanish patients with melanoma and to compare it with that of healthy controls.

PATIENTS AND METHODS

The study included 82 patients with cutaneous malignant melanoma from the Department of Dermatology, Instituto Valenciano de Oncología, and Department of Dermatology, Hospital General Universitario, Valencia, Spain. The ethics committees of these centres approved the study. All the patients and unrelated controls were of Spanish origin, from the eastern regions of the country. The diagnosis of melanoma was based on clinical and histologic evaluations. Patients were selected according to the following criteria, suggesting a possible genetic susceptibility: early age at diagnosis (≤ 30 years) and/or first-degree relatives with cancer. These criteria were used to divide the population into 4 different subgroups that were also studied separately. Median age at diagnoses was 34 years (range: 13–72). Seventy-two patients had superficially spreading melanoma, 8 had nodular melanoma, one patient had lentigo malignant melanoma and one had unclassified melanoma. Thirty-nine patients had first-degree family relatives with cancer: 5 with melanoma, one with pancreatic cancer and 33 with other non-cutaneous neoplasias. In 4 cases, family history of cancer could not be assessed and these cases were not considered for the analysis when subgroups by family history of cancer were evaluated.

 ISSN 0001-5555

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 data base 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 χ^2 analysis. Fisher's exact *p*-values were calculated for analyses in which one or more variables within 2×2 tables were less than 5. The Bonferoni method was used to correct for the number of comparisons (P_c), 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 ≤ 30 years (9.4 versus 25%; $p = 0.06$; $p_c = 0.42$; RR = 0.31), 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 of cancer 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 I. *Frequencies of HLA DQA1 alleles*

			Age at diagnosis				Family history of cancer[a]					
Alleles	Melanoma cases		Age ≤ 30		Age > 30		Yes		No		Controls	
HLA-DQA1*	*n* = 82	(%)	*n* = 32	(%)	*n* = 50	(%)	*n* = 39	(%)	*n* = 39	(%)	*n* = 367	(%)
0101	13	(16)	5	(16)	8	(16)	8	(21)	5	(13)	85	(3)
0102	25	(31)	10	(31)	15	(30)	15	(39)	10	(26)	102	(23)
0103	17	(21)	7	(22)	10	(20)	5	(13)	11	(28)	62	(17)
0104	2	(2)	0	(0)	2	(4)	1	(3)	1	(3)	27	(7)
0105	2	(2)	1	(3)	1	(2)	0	(0)	1	(3)	10	(3)
0201	22	(27)	10	(31)	12	(24)	10	(26)	11	(28)	103	(28)
03011	11	(13)	3	(9)	8	(16)	5	(13)	5	(13)	61	(17)
0302	3	(4)	1	(3)	2	(4)	1	(3)	1	(3)	6	(2)
0303	5	(6)	1	(3)	4	(8)	3	(8)	1	(3)	25	(7)
0401	5	(6)	1	(3)	4	(8)	0	(0)	4	(11)	26	(7)
0501	24	(29)	10	(31)	14	(28)	10	(26)	13	(33)	79	(22)
0505	22	(27)	9	(28)	13	(26)	11	(28)	10	(26)	98	(27)

[a] Missing data in 4 cases.

Table II. *Frequencies of HLA DQB1 alleles*

Alleles	Melanoma cases		Age at diagnosis: Age ≤ 30		Age at diagnosis: Age > 30		Family history of cancer[a]: Yes		Family history of cancer[a]: No		Controls	
HLA-DQB1*	$n = 82$	(%)	$n = 32$	(%)	$n = 50$	(%)	$n = 39$	(%)	$n = 39$	(%)	$n = 100$	(%)
05	22	(27)	8	(25)	14	(28)	13	(33)	8	(21)	37	(37)
06	34	(42)	13	(41)	21	(42)	16	(41)	20	(51)	37	(37)
02	42	(51)	18	(56)	24	(48)	19	(49)	21	(54)	46	(46)
0301/0309/0310	23	(28)	9	(28)	14	(28)	11	(28)	10	(26)	31	(31)
0302/0307/0308	12	(15)	3	(9)	9	(18)	5	(13)	6	(15)	25	(25)
0303/0306	5	(6)	1	(3)	4	(8)	3	(8)	2	(5)	4	(4)
04	6	(7)	1	(3)	5	(10)	1	(3)	4	(10)	3	(3)

[a] Missing data in 4 cases.

Table III. *Frequencies of HLA DRB1 alleles*

Alleles	Melanoma cases		Age at diagnosis: Age ≤ 30		Age at diagnosis: Age > 30		Family history of cancer[a]: Yes		Family history of cancer[a]: No		Controls	
LA-DRB1*	$n = 82$	(%)	$n = 32$	(%)	$n = 50$	(%)	$n = 39$	(%)	$n = 39$	(%)	$n = 367$	(%)
0101/0102/0104–0106	12	(15)	4	(13)	8	(16)	8	(21)	4	(10)	74	(20)
0103	3	(4)	2	(7)	1	(2)	0	(0)	3	(8)	12	(3)
1501–1509	21	(26)	9	(28)	12	(24)	11	(28)	9	(23)	73	(20)
1601–1608	4	(5)	1	(3)	3	(6)	3	(8)	1	(3)	9	(2)
0301/0304–0316	24	(29)	10	(31)	14	(28)	10	(26)	13	(33)	78	(21)
0302/0303	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	2	(1)
04	16	(20)	4	(13)	12	(24)	8	(21)	6	(15)	88	(24)
11	16	(20)	8	(25)	8	(16)	8	(21)	7	(18)	83	(23)
12	3	(4)	1	(3)	2	(4)	2	(5)	1	(3)	6	(2)
13	20	(24)	7	(22)	13	(26)	7	(18)	13	(33)	92	(25)
14	2	(2)	0	(0)	2	(4)	1	(3)	1	(3)	27	(7)
07	21	(26)	9	(28)	12	(24)	9	(23)	11	(28)	104	(28)
08	5	(6)	1	(3)	4	(8)	0	(0)	4	(10)	23	(6)
09012	3	(4)	1	(3)	2	(4)	2	(5)	1	(3)	6	(2)
1001	2	(2)	1	(3)	1	(2)	0	(0)	1	(3)	6	(2)

[a] 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 Bonferoni 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

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.

ACKNOWLEDGEMENTS

We thank Ken Martin for his invaluable help in correcting the English language style of the manuscript. This investigation was partly supported by grant GV-AE00-07 from the Conselleria d'Educació i Ciencia of the Generalitat Valenciana.

REFERENCES

1. Cabrera T, Ruiz-Cabello F, Garrido F. Biological implications of HLA-DR expression in tumours. Scand J Immunol 1995; 41: 398–406.
2. Zöller M, Strubel A, Hammerling G, Andrighetto G, Raz A, Ben-Ze'ev A. Interferon gamma treatment of B16 melanoma cells: opposing effects for non-adaptative and adaptative immune defense and its reflection by metastatic spread. Int J Cancer 1988; 41: 256–66.
3. Bouwes Bavink JN, Claas FHJ. The role of HLA molecules in the development of skin cancer. Hum Immunol 1994; 41: 173–9.
4. Le Poole IC, Mutis T, van den Wijngaard RM, Westerhof W, Ottenhoff T, de Vries RR, et al. A novel, antigen-presenting function of melanocytes and its possible relationship to hypopigmentary disorders. J Immunol 1993; 15: 7284–92.
5. Barger BO, Acton RT, Soong SJ, Roseman J, Balch C. Increase of HLA-DR4 in melanoma patients from Alabama. Cancer Res 1982; 42: 4276–9.
6. Mueller-Eckhardt G, Schendel DJ, Hundeiker M, Riedel T, O'Neill GJ, Riethmuller G, et al. Possible association between HLA-DR5 and superficial spreading malignant melanoma. Int J Cancer 1984; 34: 751–5.
7. Walker GJ, Nancarrow DJ, Walters MK, Palmer JM, Weber JL, Hayward NK. Linkage analysis in familial melanoma kindreds to markers on chromosome 6p. Int J Cancer 1994; 59: 771–5.
8. Lee JE, Loflin PT, Laud PR, Lu M, Reveille JD, Lawlor DA. The human leukocyte antigen TAP2 gene defines the centrometric limit of melanoma susceptibility on chromosome 6p. Tissue Antigens 1996; 47: 117–21.
9. Lee JE, Reveille JD, Ross MI, Platsoucas CH. HLA-DQB1-0301 association with increased cutaneous melanoma risk. Int J Cancer 1994; 59: 510–3.
10. Bateman AC, Turner SJ, Theaker JM, Howell WM. HLA-DQB1*0303 and *0301 alleles influence susceptibility to and prognosis in cutaneous malignant melanoma in the British Caucasian population. Tissue Antigens 1998; 52: 67–73.
11. Lombardi ML, Mercuro O, Pirozzi G, Ionna F, Lombari V, Mozzillo N, et al. Molecular analysis of HLA DRB1 and DQB1 polymorphism in Italian melanoma patients. J Immunother 1998; 21: 435–9.
12. Lulli P, Grammatico P, Brioli G, Catricala C, Morellini M, Roccella M, et al. HLA-DR and -DQ alleles in Italian patients with melanoma. Tissue Antigens 1998; 51: 276–80.
13. Kageshita T, Naruse T, Hirai S, Ono T, Horikoshi T, Nakagawa H, et al. Molecular genetic analysis of HLA class II alleles in Japanese patients with melanoma. Tissue Antigens 1997; 49: 466–70.
14. Kawaski ES. Sample preparation from blood, cells, and other fluids. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide methods and application. New York: Academic Press, 1990: 146.
15. Olerup O, Zetterquist H. HLA DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992; 39: 225.
16. Olerup O, Aldener A, Fogdell A. HLA-DQB1 and -DQA1 typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. Tissue Antigens 1993; 41: 119.
17. Tiwari JL, Terasaki PI. The data and statistical analysis. In: Tiwari JL, Terasaki PI, eds. HLA and disease associations. New York: Springer-Verlag, 1985: 18.
18. Woolf B. On estimating the relationship between blood group and disease. Ann Hum Genet 1955; 19: 251–3.