UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

FACULTAD DE MEDICINA

DIVISION DE ESTUDIOS DE POSGRADOS E INVESTIGACION

HLA STUDY ON TWO MEXICAN MESTIZO FAMILIES WITH SUTOIMMUNE THYROID DISEASE

T E S I S

QUE PARA OBTENER EL TITULO DE:
MEDICINA INTERNA

P R E S E N T A :
DRA. BETINA HERNANDEZ MARTINEZ

2003

FACULTAD DE MEDICINA
DIVISION DE ESTUDIOS DE POSGRADO E INVESTIGACION

SUBDIVISION DE ESPECIALIZACIONES MEDICAS

OFICIO FMED/SEM/1524/2003

ASUNTO: Autorización del trabajo de investigación
De la Dra. Betina Hernández Martínez

DR.ISIDRO AVILA MARTINEZ
SECRETARIO DE SERVICIOS ESCOLARES
DE LA FACULTAD DE MEDICINA
Presente.

Estimado Dr.Avila Martínez:

Me permito informar a usted que la **Dra. Betina Hernández Martínez** alumno del curso de especialización en Medicina Interna en Medica Sur., presenta el trabajo de investigación intitulado **"HLA STUDY ON TWO MEXICAN MESTIZO FAMILIES WITH AUTOIMMUNE THYROID DISEASE".**

De conformidad con el artículo 21 capítulo 5°. de las Normas Operativas del Plan Unico de Especializaciones Médicas (PUEM) se considera que cumple con los requisitos para validarlo como el trabajo formal de Investigación que le otorga el derecho a la diplomación como especialista.

Sin otro particular de momento, reciba un cordial saludo.

Atentamente
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D. F. a 27 de agosto del 2003

JEFE DE LA SUBDIVISION

DR. LEOBARDO C. RUIZ PEREZ

LRP*mjt.

Médica Sur

Agosto 26, 2003

Dr. Leobardo C. Ruiz Pérez
Jefe de la Subdivisión de Especializaciones
Médicas.
Facultad de Medicina
División de Estudios de Posgrado e Investigación.

Estimado Dr. Ruiz:

Por medio de la presente me permito informarle que después de una exhaustiva revisión por parte de nuestro Comité de Enseñanza y de la Dirección Académica no existe inconveniente para que la **Dra. Betina Hernández Martínez**, residente del cuarto año de la Especialidad de Medicina Interna pueda titularse en forma oportuna con el artículo publicado en la Revista Autoimmunity en el 2002 vol 35(4): 265-269 en donde participó como coautora de este trabajo titulado:

"HLA study on two Mexican mestizo families with autoimmune thyroid disease"

Sin más por el momento y agradeciendo su apoyo, le envío un cordial saludo y quedo a sus órdenes para cualquier o aclaración.

Atentamente,

Dr. Javier Lizardi Cervera
Subdirector Académico
Profesor Adjunto del Curso de
Especialización en Medicina Interna

Autoimmunity, 2002 Vol. 35 (4), pp. 265–269

Taylor&Francis
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HLA Study on Two Mexican Mestizo Families with Autoimmune Thyroid Disease

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(Submitted 31 May 2001; Accepted with revisions 12 March 2002)

Several studies have been done regarding the genetic susceptibility to autoimmune thyroid disease, particularly those related to the role of Major Histocompatibility Complex (MHC) genes in the etiology of the disease.

In the present study, we report class I and class II MHC haplotypes in nine individuals affected by Hashimoto thyroiditis and Graves' disease who belong to two distinct Mexican families.

In one of the families, Hashimoto thyroiditis was associated with the Human Leukocyte Antigen (HLA) HLA-DR3 allele whereas in the other family the disease was associated with homozygosity for the HLA-DR4 (DRB1*0407), HLA-DQ3 (DQB1*0302) haplotype. On the other hand, Graves' disease was found to be associated in one of the families with HLA-DR2 (DRB1*1501) and in the other with homozygosity for the HLA-DR7 (DRB*0701) and HLA-DQ2 (DQB1*0201) haplotype.

These results confirm that in Mexicans as in other ethnic groups, genes located within the MHC region are related to the genetic susceptibility to develop autoimmune thyroid disease.

Keywords: Hashimoto; Graves; Autoimmune; Thyroiditis; HLA; Mexican

INTRODUCTION

There are many autoimmune syndromes with common immune features. The autoimmune thyroid disease is characterized by the production of autoantibodies against different thyroidal antigens such as thyroglobulin (Tg), thyroid stimulant hormone receptor (TSHr) and thyroidal peroxidase.[1] The autoimmune thyroid disease includes three different clinical entities: (1) Hashimoto thyroiditis; (2) Graves-Basedow disease and (3) Post partum thyroiditis. Thyroid disease is mainly determined by environmental and genetic factors. The class II genes of Major Histocompatibility Complex (MHC) are located on the short arm of the sixth chromosome (Fig. 1). In transgenic mice, an important role for the Human Leukocyte Antigen (HLA-DR3) has been demonstrated in the susceptibility to Hashimoto's thyroiditis as well as the importance of Tg as a thyroid autoantigen.[2] Hashimoto's thyroiditis is also common in families with insulin-dependent diabetes mellitus which may be due, in part, to common disease susceptibility genes. In these families, genetic analyses have revealed a two-fold increase in DQA1*0501-DQB1*0201 among the Hashimoto's compared to non-Hashimoto's haplotypes in Caucasians.[3] Alleles of these genes have been associated with the development of this disease in different ethnic groups. Hashimoto's thyroiditis is associated with HLA-DR5 and HLA-DQA1*0501 in Caucasian patients;[4] HLA-DR3, DR7 and DQ2 in British patients;[5,6] HLA-DR9 in Chinese patients[7] and the antigen DRw53 in Japanese patients.[8] On the other hand, Graves-Basedow's disease has been strongly associated with HLA-B8, DR3 and DR17 alleles in white European patients[6] and with HLA-DRB1*0803 and DRB1*1403 in Asian patients.[9,10] In African-American women, HLA-DR and DQ seldom contribute to the development of this disease.[11]

In Mexican Mestizos, the strong genetic influence of Amerindian genes with admixture estimates have shown a proportion of 56% Amerindian genes, 40% white genes and up to 4% black genes.[12–14] Mexican Mestizos are therefore a suitable group to study the role of ethnicity in the susceptibility to develop autoimmune thyroid disease.

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ISSN 0891-6934 print/ISSN 1607-842X online © 2002 Taylor & Francis Ltd
DOI: 10.1080/08916930210000010712

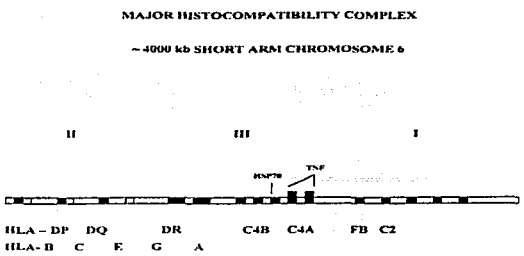

FIGURE 1 MHC map located at the short arm of the human chromosome 6.

The main objective of this study was to find the distribution of MHC class II genes and its haplotypes in nine individuals of two Mexican multicase families in which several of its members are affected with autoimmune thyroid disease.

PATIENTS AND METHODS

Subjects

Nine members of two Mexican Mestizo families with autoimmune thyroid disease (Hashimoto's thyroiditis, Graves-Basedow's disease) were studied. A Mexican Mestizo is defined as a person who was born in Mexico and whose last two ascending generations were also born in Mexico. Admixture estimates have shown a proportion of 56% Amerindian genes, 40% white genes and up to 4% black genes.[12-14] All patients were from the Thyroid Clinic at the Insituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubiran. Family A had autoimmune thyroiditis and family B had Graves' disease. Members of the two Mexican families were followed in our Thyroid outpatient clinic. We acquired clinical information by routine history (past and present complaints) and a directed questionnaire regarding symptoms related to thyroid disease. A full clinical examination was performed at the time of this visit as well.

Autoimmune thyroid disease was diagnosed by patient history and clinical examination, confirmed by thyroid function tests and thyroid autoantibodies measurement.

HLA Class I and II Typing

Peripheral blood mononuclear cells were isolated by the density gradients centrifugation technique and B cells were isolated with the Lympho-Kwik (One Lambda, California USA) reagent. The serologic HLA-A, B and C antigen typing was done in peripheral blood mononuclear cells, while for HLA-DR and DQ typing, B lymphocytes were used. Both determinations were performed by the microlymphocytotoxicity method. A 210 antisera panel was used (C-Six Diagnostics, Mequon, WI, USA), 140 to define HLA-A, B and C specificities and 70 to define 27 HLA-DR and DQ specificities. Genomic DNA was isolated by the Salting Out procedure from peripheral blood mononuclear cells (Maxim Biotech, Inc., BDtract Genomic DNA Isolation Kit, San Francisco, CA, USA). Generic HLA-A, B and DR genes were determined by PCR-SSP (Pel.Freez, Brown Deer, WI, USA) and electrophoresis in 2% agarose gel stained with ethidium bromide. HLA-DRB1 and HLA-DQB1 subtypes were determined by a Dot blot hybridization procedure using allele specific oligonucleotides (ASO). Information of the sequences and specificities of the DRB1, DRB3, DRB5 and DQB1 oligonucleotides is from the 12th International Histocompatibility Workshop.

RESULTS

Figure 2 shows family A in which three sisters were affected from Hashimoto's thyroiditis (II.2, II.3, II.4). Interestingly, two of them (II.2 and II.3) have the HLA-A10, B21, Cw6, DRB1*0301, DQB1*0301 haplotype and all three sisters expressed the HLA-DR3; the homologous chromosome is different since the II.5 patient has the HLA-A2, B5, Cw6, DR2, DQ1, DRw53 haplotype. The brothers II.6 and II.7 have the HLA-A28, B62, Cw3, DR6, DQ3, DRw52 haplotype and they also showed HLA-DR3 as a part of the extended haplotype Cw7, DR3, DQ2, DRw52. In addition, it is important to notice that two of the patients with Graves-Basedow's disease (I.2, II.5) share the HLA-A2, B5, Dw6, DR2, DQ1, DRw53 haplotype suggesting a direct association between this haplotype and Graves-Basedow's disease.

Figure 3 shows family B wherein the mother (I.2) was affected by Grave-Basedow's disease and both haplotyes (HLA-A19, B5, Cw1, DR4, DQ3, DRw53 and A10, B12, Cw3, DR7, DQ2, DRw53) have been segregated in her four daughters (II.2, II.3, II.5, II.7) who have Graves' disease, suggesting that both haplotypes are involved. Furthermore, patients II.3 and II.5 are homozygous for haplotype DRB1*0701, DQB1*0201 and DRw53.

DISCUSSION

This study describes two Mexican families with thyroid autoimmune disease. Hashimoto's thyroiditis was associated in one of the families with HLA-DR3 allele and in the other family with homozygocity for the HLA-DR4, DQ3, DRw53 haplotype.

Graves-Basedow's disease was associated with HLA-DR2 allele in family A and with homozygocity for HLA-DRB1*0701, DQB1*0201, DRw53 haplotype in family B. This data suggests that MHC class II genes are associated with the development of thyroid autoimmune disease in Mexican Mestizo individuals.

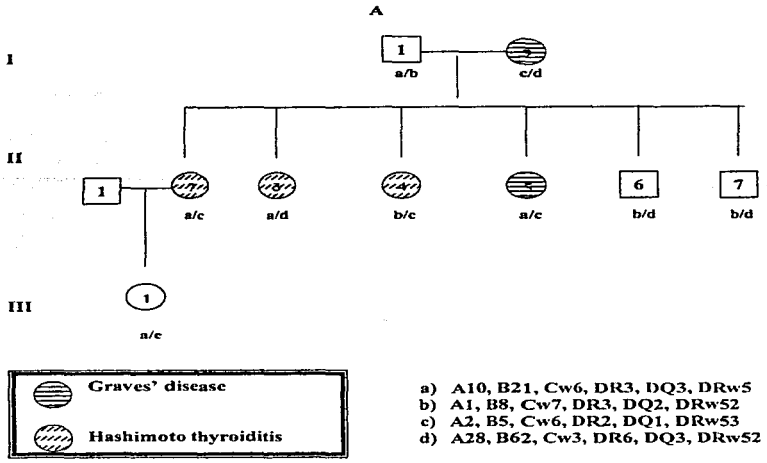

FIGURE 2 Shows the family pedigree (family A) with autoimmune thyroid disease, in which the mother and a daughter suffer from Graves-Basedow disease (I.2, II.5) and all the three daughters (II.2, II.3, II.4) suffer from Hashimoto thyroiditis. Besides, it can be appreciated the segregation of extended haplotypes of MHC in each one of the individuals.

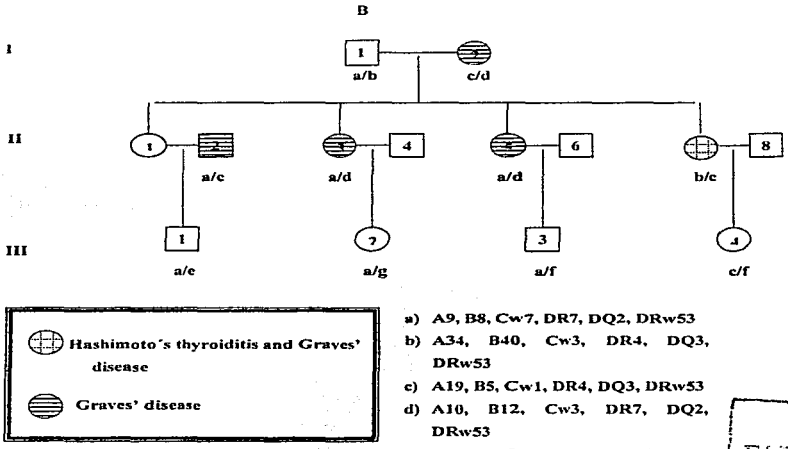

FIGURE 3 Shows the pedigree of a family (family B) with multiple autoimmune disease, where it can be appreciated the haplotypes of MHC in the mother (I.2) and the daughters affected by Graves-Basedow disease (II.2, II.3, II.5, II.7).

The results in family A confirm an association described previously between autoimmune thyroiditis and HLA-DR3.[12] In family B, the association between autoimmune thyroiditis agrees with the results found in studies where susceptibility appears to be mediated through either HLA-DQB*0201 or DQA*0301.[15,16]

Some studies have shown association of HLA-DR genes with Hashimoto's thyroiditis in different populations. The disease is differentially associated with HLA-DR5 and HLA-DQA1*0501[8] in Caucassian populations, with HLA-DRw53 and HLA-DRB1*0803[18,19] in Japanese populations and with the extended haplotype HLA-B46, DR9 in Southern Chinese populations, which seems to be equivalent with HLA-B8, DR3 in Caucasians. In the Chinese group, the HLA-B17 allele was found to be increased in patients with goiter.[17] Canadian,[17,18] English,[19,20] Hungarian,[21] Swedish,[22] French[23] and German[24] studies showed association between Graves-Basedow's disease and HLA-B8 and DR3 alleles. However, studies in Asian patients showed different results; in Japanese patients, the disease is associated with HLA-DR5 and DR8 (DRB1*0803)[25,26] and in Korean patients, with HLA-B13, DR5 and DR8.[9]

In South African patients, Graves-Basedow's disease is associated with HLA-DR1 and in Indian patients with HLA-B8 and DQ2.[27,28]

As observed in family A, Hashimoto's thyroiditis patients seem to have combined susceptibility: on one hand, a Caucasian gene (DR3) and on other, an Asian gene (DRw53). The same explanation can be given for family B.

Interestingly, the main haplotypes involved in autoimmune thyroid disease are very rare in normal Mexican populations,[29-32] which suggests that the alleles involed with Graves' disease and Hashimoto's thyroiditis in both families are not autochtonous. This is probably the result of breeding with other ethnic groups, such as Caucasian or Asian, including the association with homozygocity for HLA-DR7, DQ2, DRw53 segment of family B, which has not been described yet.

We have found an association of systemic lupus erythematosus (SLE) with HLA-DR3 in Mexican Mestizo patients. There is a subgroup of patients affected by SLE in which exists an association between the production of anticardiolipin antibodies, the development of the antiphospholipid syndrome and HLA-DR7. This suggests that autoimmunity in Mexicans is strongly related to the presence of these antigens, possibly acquired through breeding with other ethnic groups.[29-32]

It is important to mention that HLA-A1, B8, Cw7, DR3, DQ2, DRw52 haplotype(Ab) is from Caucasian origin, whereas HLA-A2, B5, CW6, DR2, DQ1, DRw53 haplotype(Ac) has Asian origin. Both are associated with the genetic susceptibility to develop SLE in Mexican Mestizo patients.[32] Ab has also been associated with type I diabetes mellitus in patients of European ancestry.[33]

Haplotype HLA-A10, B12, Cw3, DR7, DQ2, DRw53 is from Italian population, and is associated with celiac disease in patients with Jewish ancestry;[34] is very rare in healthy Mexican Mestizo population.[31] Finally HLA A-28, B62, Cw3, DR6, DQ3, DRw52 and HLA-A28, B12, Cw6, DR6, DQ1, DRw52 are autochtonous. Both are present in healthy Mexican Mestizo population in 15%.[31]

The first Amerindian Natives are believed to come from Asia trough the Bering land bridge between 30,000 and 12,000 years before the present. These conclusions have been based on cultural, morphological, and genetic similarities between American and Asian populations.[35]

CONCLUSION

This study confirms that the genetic susceptibility to develop Hashimoto's thyroiditis is strongly associated with alleles related with the specificity of HLA-DRw53. It is very unlikely that the susceptibility came from breeding with Oriental genes. The autoimmune thyroid diseases are mainly related with HLA class II genes and probably the specific gene responsible for autoimmune thyroid processes is pleiotropic and possesses numerous variants particular for each ethnic group. Finally, the homozygocity for MHC haplotypes suggests that the susceptibility mentioned seems to be inherited with an autosomical recessive pattern.

References

[1] Kotani, T., Umeki, K., Matsunaga, S., Kato, E. and Ohtaki, S. (1986) "Detection of autoantibodies to thyroid peroxidase in autoimmune thyroid diseases by micro-ELISA and immunoblotting", *J. Clin. Endocrinol. Metab.* **62**, 928–933.

[2] Kong, Y.M., Lomo, L., Motte, R.W., Giraldo, A.A., Baisch, J., Strauss, G., Hämmerling, G. and David, Ch. (1996) "HLA-DRB1 polymorphism determines susceptibility to autoimmune thyroiditis in transgenic mice: definitive association with HLA-DRB1*0301 (DR3) gene", *J. Exp. Med.* **184**, 1167–1172.

[3] Dorman, J., Kramer, M.K., O'Lear, L.A., Burke, J.P., McCanlies, E., Mc Carthy, B., Trucco, M., Swan, J.S., Steenkiste, A.R., Koehler, A.N. and Foley, T.P. (1997) "Molecular epidemiology of autoimmune thyroid disease", *Gac. Méd. Méx.*(Suppl. 1), 97–103.

[4] Weissel, M., Hofer, R., Zasmeta, H. and Mayr, W.R. (1980) "HLA-DR and Hashimoto thyroiditis", *Tissue Antigens* **160**, 302–310.

[5] Tandon, N., Zhang, L. and Weetman, A.P. (1991) "HLA associations in Hashimoto thyroiditis", *Clin. Endocrinol.* **34**, 383–386.

[6] Ratanachaiyavong, S. and McGregor, A.M. (1994) "HLA-DPB1 polymorphisms on the MHC extended haplotypes of families of patients with Graves' disease: two distinct HLA-DR17 haplotypes", *Eur. J. Clin. Investig.* **25**, 309–315.

[7] Hawkins, B.R., Ma, J.T.C., Lam, K.S.L., Wang, C.C.L. and Yeung, R.T.T. (1987) "Strong association between HLA-DRw9 and Hashimoto thyroiditis in Southern Chinese", *Acta Endocrinol.* **114**, 543–546.

[8] Gorski, J., Rollini, P. and Mach, B. (1987) "Structural comparison of the genes of two HLA-DR supertypic groups: the loci encoding DRw52 and DRw53 are not truly allelic", *Immunogenetics* **25**, 387–402.

[9] Cho, B.Y., Rhee, B.D., Lee, D.S., Lee, M.S., Kim, G.Y., Lee, H.K., *et al.* (1987) "HLA and Graves' disease in Koreans", *Tissue Antigens* **31**, 119–121.

[10] Katsuren, E., Awata, T., Matrumoto, C. and Yamamoto, K. (1994) "HLA class II alleles in Japanese patients with Graves' disease:

weak associations of HLA-DR and DQ", *Endocrinol. J.* **41**, 599–603.

[11] Yanagawa, T. and De Groot, L.J. (1996) "HLA class II associations in African-American female patients with Graves' disease", *Thyroid* **6**, 37–39.

[12] Lisker, R., Pérez-Briseño, R., Granados, J., *et al.* (1988) "Gene frequencies and admixtures estimates in a Mexico City population", *Am. J. Phys. Anthropol.* **71**, 203–207.

[13] Lisker, R., Pérez-Briseño, R., Granados, J., *et al.* (1988) "Gene frequencies and admixtures estimates in the state of Puebla, Mexico", *Am. J. Phys. Anthropol.* **76**, 331–335.

[14] Lisker, R., Ramírez, E., Pérez-Briseño, R., *et al.* (1990) "Gene frequencies and admixtures estimates in four Mexican urban centers", *Hum. Biol.* **62**, 791–801.

[15] Shi, Y., Minjing, Z., Desmond, R. and Farid, N. (1988) "Typing for major histocompatibility complex class II antigens in thyroid tissue blocks: association of Hashimoto's thyroiditis with HLA-DQA*0301 and DQB*0201 alleles", *J. Clin. Endocrinol. Metab.* **66**(2), 367–375.

[16] Farid, N.R., Stone, E. and Johnson, G. (1980) "Graves' disease and HLA clinical and epidemiological associations", *Clin. Endocrinol.* **13**, 535–544.

[17] Frecker, M., Mercer, G., Skanes, V.M. and Farid, N.R. (1988) "Major histocompatibility complex (MHC) factors predisposing to and protecting against Graves' eye disease", *Autoimmunity* **1**, 307–315.

[18] Frecker, M., Stenszky, V., Balázs, C., Kozma, L., Kraszits, E. and Farid, N.R. (1986) "Genetic factors in Graves' ophtalmopathy", *Clin. Endocrinol.* **25**, 479–485.

[19] Weetman, A.P., So, A.K., Warner, C.A., Foroni, L., Fells, P. and Shine, B. (1988) "Immunogenetics of Graves' ophtalmopathy", *Clin. Endocrinol.* **28**, 619–628.

[20] Kendall-Taylor, P., Stephenson, A., Stratton, A., Papiha, S.S., Perros, P. and Roberts, D.F. (1988) "Differentiation of autoimmune ophtalmopathy from Graves' hyperthyroidism by analysis of genetic markers", *Clin. Endocrinol.* **28**, 601–610.

[21] Allannic, H., Fauchet, R., Lorcy, Y., Gueguen, M., LuGuerrier, A.M. and Genetet, B. (1983) "A prospective study of the relationship between relapse of hyperthyroid Graves' disease after antithyroid drugs and HLA haplotype", *J. Clin. Endocrinol. Metab.* **57**, 719–722.

[22] Dahlberg, P.A., Holmlund, G., Karlsson, F.A. and Säfwenberg, J. (1981) "HLA-A, B, C and DR antigens in patients with Graves' disease and their correlation with signs and clinical course", *Acta Endocrinol.* **97**, 42–47.

[23] De Bruin, T.W.A., Patawardhan, N.A., Brown, R.S. and Braverman, L.E. (1988) "Graves' disease: changes in TSH receptor and anti-microsomal antibodies after thyroidectomy", *Clin. Exp. Immunol.* **72**, 481–485.

[24] Badenhoop, K., Walfish, P.G., Rau, H., Fisher, S., Nicolay, A., Bogner, U., *et al.* (1995) "Susceptibility and resistance alleles of human leukocyte antigen HLA-DQA1 and HLA-DQB1 are shared in endocrine autoimmune disease", *J. Clin. Endocrinol. Metab.* **80**, 2112–2117.

[25] Tamai, H., Sudo, T., Kimura, A., Mikuta, T., Mutsubayashi, S., Kuma, K., *et al.* (1997) "Association between the DRB1*08032 histocompatibility antigen and methimazole-induced agranulocytosis in Japanese patients with Graves' disease", *Ann. Intern. Med.* **124**, 490–494.

[26] Sasanaki, T., Nishimura, Y., Muto, M. and Otha, N. (1983) "HLA-linked genes controlling immune response and disease susceptibility", *Immunol. Rev.* **70**, 51–73.

[27] Omar, M., Hammomnd, M.G., Desai, R.K., Motala, A.A., Aboo, N. and Seedat, M.A. (1990) "HLA class I and II antigens in South African blacks with Graves' disease", *Clin. Immunol. Immunopathol.* **54**, 98–102.

[28] Tandon, N., Mehra, N.K., Taneja, V., Vaidya, M.C. and Kochupillai, N. (1990) "HLA antigens in Asian Indian patients with Graves' disease", *Clin. Endocrinol.* **33**, 21–26.

[29] Granados, J., Vargas-Alarcón, G., Drenkard, C., Andrade, F., Melín-Aldana, H., Alcocer-Varela, J. and Alarcón-Segovia, D. (1997) "Relationship of anticardiolipin syndrome to HLA-DR7 in Mexican patients with systemic lupus erythematosus (SLE)", *Lupus* **6**, 57–62.

[30] Weckmann, A.L., Vargas-Alarcón, G., López, M., González, N., De Leo, C., Castelán, N., Bordes, J., *et al.* (1996) "Frequencies of HLA-A and HLA-B alleles in a Mexico City Mestizo group", *Am. J. Hum. Biol.* **9**, 1–5.

[31] De Leo, C., Castelán, N., López, M., González, N., Weckmann, A.L., Melín-Aldana, H., Vargas-Alarcón, G., Bordes, J., Alarcón-Segovia, D., Granados, J., Ramírez, E. and Lisker, R. (1997) "HLA class I and class II alleles and haplotypes in Mexican Mestizos established from serological typing of fifty families", *Hum. Biol.* **69**(6), 809–818.

[32] Granados, J., Vargas-Alarcón, G., Andrade, F., Melín-Aldana, H., Alcocer-Varela, J. and Alarcón-Segovia, D. (1996) "The role of HLA-DR alleles and complotypes through the ethnic barrier in Mexican SLE patients", *Lupus* **5**, 184–189.

[33] Deschamps, I., Marcelli-Barge, A., Poirier, J.C., *et al.* (1988) "Two distinct HLA-DR3 haplotypes are associated with age related heterogeneity in type 1 diabetes", *Diabetologia*, 896–901.

[34] Sollid, L.M. and Thorsby, E. (1993) "HLA susceptibility genes in celiac disease: genetic mapping and role in pathogenesis", *Gastroenterology* **105**, 910–922.

[35] Arnaiz-Villena, A., Vargas-Alarcón, G., Granados, J., *et al.* (2000) "HLA genes in Mexican Mazatecans, the peopling of the Americas and the uniqueness of Amerindians", *Tissue Antigens* **56**, 405–416.