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Extended major histocompatibility complex haplotypes, ancestry and acute kidney transplant rejection in Mexicans

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ABSTRACT

Introduction. Extended major histocompatibility complex (MHC) haplotypes are associated with several autoimmune diseases, and these appear to depend on ancestry. **Objective.** To evaluate the association of extended MHC gene frequencies, ancestry, and acute rejection. Material and methods. 127 living kidney transplant recipients who underwent kidney transplantation in Mexico City between January 2004 and October 2007 with follow up until October 2008. The primary outcome was biopsy proven acute rejection. Ancestry was considered as either Amerindian or admixtures with Caucasian, African or Oriental genes. Allele and haplotype frequencies were estimated for HLA A, B and DR loci. Hardy Weinberg (HW) and delta values were analyzed to test for linkage disequilibrium (LD). Results. There were no significant differences in the baseline characteristics between groups. 50% were men, and 28, 61 and 10% of the patients shared zero, one or two haplotypes, respectively. The whole population was Hispanic and born in Mexico. Median PRA was 0%. Allelic variance in all MCH loci was in HW equilibrium, 14% developed acute rejection. There was a high frequency of Amerindian haplotypes; admixture genes and LD were higher in the group with acute rejection. When compared to the group without acute rejection, the haplotype A1*B8*DR3 was more frequent in donors in whom their recipients had acute rejection (p = 0.008), while A28*B39*DR4 was more common in the recipients with acute rejection (p = 0.003). Multivariate Cox regression models did not attenuate these associations. **Conclusions.** Ancestry and LD may be associated with risk of acute rejection and may therefore be useful in directing immunosuppression.

Haplotipos extendidos del complejo mayor de histocompatibilidad, ancestría y rechazo renal agudo en pacientes mexicanos trasplantados

RESUMEN

Introducción. Los haplotipos extendidos del complejo mayor de histocompatibilidad (CMH) se asocian con diversas enfermedades autoinmunes y éstas parecen ser dependientes de la ancestría. ***Objetivo.*** *Evaluar la asociación entre las frecuencias génicas de los genes del CMH, la ancestría y el rechazo agudo. Material y métodos. 127 receptores de donador vivo sometidos a trasplante renal en la Ciudad de México entre enero 2004 hasta octubre 2007 con seguimiento hasta octubre 2008. El objetivo primario fue la frecuencia de rechazo agudo confirmado por biopsia renal. Ancestría se consideró ya sea como amerindios o mezcla génica entre caucásicos, africanos o genes orientales. Las frecuencias alélicas y haplotipos se estimaron para los loci HLA A, B y DR. Hardy Weinberg (HW) y valores delta se analizaron para comprobar el desequilibrio de ligamiento (LD). Resultados. No hubo diferencias significativas en las características basales entre los grupos; 50% fueron hombres y 28, 61 y 10% de los pacientes compartieron cero, uno o dos haplotipos, respectivamente. Toda la población fue hispánica y nacida en México. La media del porcentaje reactivo de anticuerpos (PRA) fue 0%. La variabilidad en todos los loci del CMH se encontró en equilibrio de Hardy Weinberg; 14% desarrollaron rechazo agudo. Hubo una frecuencia elevada de haplotipos amerindios. La mezcla de los genes y el desequilibrio de linaje (DL) fueron más altos en el grupo de rechazo agudo. Cuando se comparó con el grupo sin rechazo agudo, el haplotipo*

*A1*B8*DR3 se encontró con más frecuencia en los donadores cuyos receptores presentaron rechazo agudo (p = 0.008) mientras que el haplotipo A28*B39*DR4 fue más frecuente en los receptores que presentaron rechazo agudo (p = 0.003). Modelos de Regresión Multivariada de Cox no atenuaron estas asociaciones. Conclusiones. La ancestría y, el DL están asociados con un mayor riesgo de rechazo agudo y por lo tanto, pudiera ser una herramienta de utilidad para tratamientos inmunosupresores.*

Key words. Extended major histocompatibility Complex. Ancestry. Acute kidney rejection. Linkage disequlibrium. Mexican.

Palabras clave. Complejo mayor de histocompatibilidad. Ancestría. Rechazo renal agudo. Desequilibrio de ligamiento. Mexicanos.

INTRODUCTION

The analysis of HLA genes is important in the study of disease association, alloreactivity, evolution and ancestry genetics.[1] The localization of genes relevant to the major histocompatibility complex (MHC) outside the classical boundaries of this region and confirmation of extended linkage disequilibrium (LD) has led to the proposal for an extended MHC.[2]

Extended major histocompatibility complex haplotypes, which account for at least 30% of normal Caucasian haplotypes, have relatively fixed gross structure, DNA sequence and carry very similar, if not identical alleles, even when they are found in apparently unrelated individuals. Extended MHC haplotypes are defined as having strong LD amongst MHC genes.

LD is the non-random association of alleles at two or more loci in a population. LD is present when the observed haplotype distribution of two or more markers in a population is significantly different from the expected haplotype distribution (which can be derived from the cross-product of observed allele frequencies).[3]

The biological significance of LD is not completely understood, however it has been documented that extended haplotypes appear to depend on ancestry and are associated with an increased susceptibility to several autoimmune diseases.[4-6] For example, A1-B8-DR3 is one of the most common haplotypes in Northern Europe and it is related with immunologic diseases such as type 1 diabetes, celiac disease, systemic lupus erythematous and rapid HIV progression.[7]

Likewise, ancestry has demonstrated to influence the immune response by increasing the susceptibility to some diseases and response to therapeutic treatments.[8] There is limited data to support the role of LD and ancestry in the Hispanic kidney transplant population. In Mexico a complex history of genetic admixture has granted its population with a genetic diversity greater that those of pure Amerindian or Caucasian populations.[9]

OBJECTIVE

The objective of this study was to evaluate the association between allele frequencies, extended MHC haplotypes and ancestry with acute kidney transplant rejection in the Mexican population.

MATERIAL AND METHODS

Patients

Multiple center retrospective study which included 127 living renal transplant recipients who underwent kidney transplantation at the Instituto Nacional de Cardiología Ignacio Chávez and Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán between January 2004 and October 2007. Patients were followed until October 2008.

The independent variables included gene and haplotype frequencies measured by low resolution DNA typing techniques. Dependent variable was biopsy proven acute kidney rejection. The indication for performing kidney biopsy was creatinine increase > 20%.

Covariates included: age, sex, clinical site, haplotypes sharing, type of kidney donor (related *vs.* unrelated), panel of reactive antibodies (PRA), immunosuppression regimen, etiology of kidney disease, time spent in dialysis, history of diabetes, hypertension, prevalent cardiovascular disease, body mass index and systemic blood pressure.

Ancestry was considered as either autochthonous (Amerindian) or admixtures with Caucasian, African or Asian genes. A study of the HLA haplotypes was made to determine the gametic phases and the allelic, haplotypic and genotypic frequencies for

HLA-A, B and DR loci. Hardy-Weinberg (HW) equilibrium and LD were calculated with the Arlequin program (version 3.11). HW and delta values were analyzed to test for LD.

Admixture estimations were carried out and haplotype proportions were compared between recipients *vs.* recipients who presented acute transplant rejection (ATR) and donors *vs.* donors who presented ATR.

Categorical variables were analyzed by Person's Square Chi test or Fisher Exact Test. Continuous variables were analyzed by Student's T test or Mann-Whitney U test when applied. The accumulated probability free of ATR was estimated with Kaplan-Meir survival curves. Multivariate Cox regression models were performed to determine the association between haplotypes and ATR. A p value of < 0.05 was considered as statistically significant. The statistical analysis was performed using SPSS (version 14).

RESULTS

We evaluated 127 kidney transplants (254 haplotypes). Mean age for the recipients and donors was 30 and 36 years, respectively. Fifty percent (n = 64) were male, 28% (n = 36), 61% (n = 78) and 10% (n = 13) shared zero, one or two haplotypes, respectively. Fourteen percent (n = 18) of the patients presented acute rejection. The whole population was Hispanic and born in Mexico. Median PRA was zero. There were no significant differences in the baseline characteristics between the groups with and without acute rejection (Table 1). Allelic variance in all three MHC loci was in HW equilibrium.

The sample studied showed a very high heterogeneity with relatively high frequency of autochthonous (Amerindian) haplotypes. When haplotypes were divided by ancestry between autochthonous and the ones acquired through admixture with Caucasian, African or Oriental genes, we found a higher

Table 1. Population characteristics according to presence or absence of acute transplant rejection (ATR).

Variables	No ATR (N = 109)	ATR (N = 18)	P value
• Age receptor*	29.9 ± 11.4	27.1 ± 8.3	0.31
• Age donor*	35.3 ± 10.2	39.6 ± 11.0	0.11
• Time on dialysis (months)	9 (0.1-57)	5 (0.7-70)	0 27
• Basal systolic blood pressure	130 (90-180)	120 (100-170)	0.52
• Basal diastolic blood pressure	80 (58-120)	80 (58-102)	0.78
• Discharge creatinine	1.16 (0.7-2.07)	1.05 (0.8-1.40)	0.54
• PRA I	0 (0-96)	0 (0-56)	0.39
• PRA II	0 (0-93)	0 (0-97)	0.84
• Female gender	56 (51%)	7 (39%)	0.45
• Type of living donor			
- Related	95 (87.2%)	15 (83.3%)	0.71
- Non-related	14 (12.8%)	3 (16.7%)	
• Shared HLA haplotypes			
0	31 (28%)	5 (28%)	0 28
1	65 (60%)	13 (72%)	
2	13 (12%)	0 (0%)	
• Induction therapy	73 (67%)	9 (50%)	0 26
• Imunosupression regimen			
- Tacro/MMF/PDN	53 (49%)	9 (50%)	0.88
- Tacro/AZA/PDN	20 (18%)	3 (17%)	0.87
- CsA/MMF/PDN	11 (10%)	1 (5%)	0.86
- CsA/AZA/PDN	13 (12%)	5 (28%)	0.16
- Bela/MMF/PDN	11 (10%)	0 (0)	0 95
- Siro/MMF/PDN	1 (1%)	0 (0)	0 95

All values expressed in median (range). *Mean (SD).

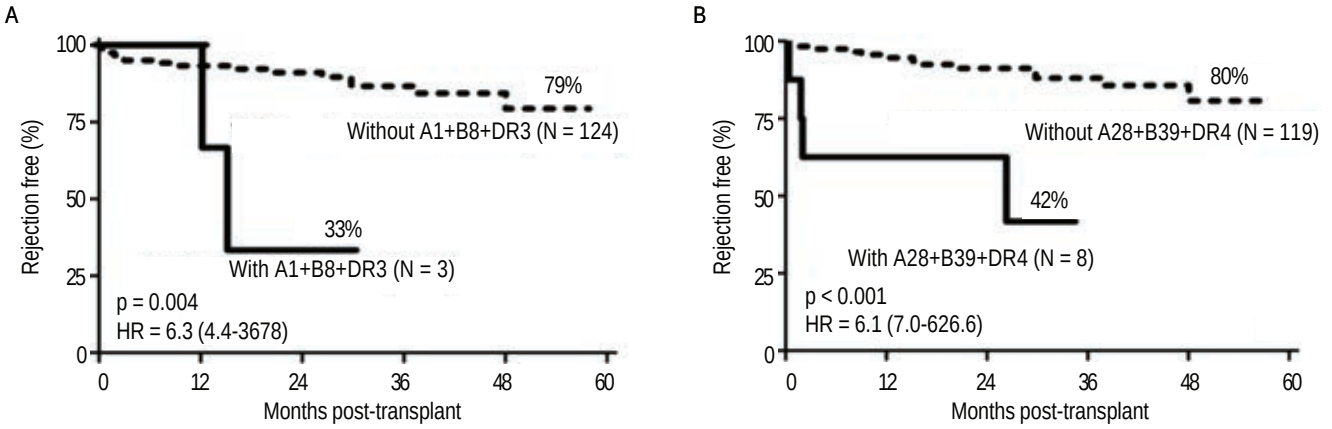

***Figure 1**. Kaplan-Meier estimated patient survival. A. In donors with HLA A1B8DR3. **B**. In receptors with HLA A28B39DR4.*

Table 2. Relationship of the haplotypes and their ethnical origin in the groups with and without ATR.

Haplotypes (A*B*DR)	1 receptor without ATR	2 donor without ATR	3 receptor with ATR	4 donor with ATR	P value	
Total	218	218	36	36	1 *vs.* 3	2 *vs.* 4
2*39*4	12	13	0	1	0.15	0.4
2*35*8	10	10	2	0	0.8	0.18
2*35*4	5	7	0	1	0.35	0.89
28*39*4	4	3	4	2	0.003	0.09
24*35*4	5	5	0	0	0.36	0.36
2*35*16	4	5	0	0	0.4	0.36
1*8*3	3	1	1	2	0.5	0.008

European proportion of genes in the donors whose recipients had acute rejection.

The haplotype HLA A1*B8*DR3 was more frequent in the donors in whom their recipients had acute rejection (p = 0.008). Likewise, the haplotype A28*B39*DR4 of Ameridian origin, was associated with acute rejection in the recipients (p = 0.003). (Figure 1).

Multivariate Cox regression models did not modify these associations (HR, 95% CI, 8.1, 1.8-37.3 and 7.1, 1.5-33.5 for haplotypes A1*B8*DR3 and A28*B39*DR4, respectively). There was a higher percentage of extended haplotypyes in ATR group and this as associated with a risk for ATR (Table 2).

DISCUSSION

In the population studied, we found two extended MHC haplotypes associated with ATR. These haplotypes showed strong LD and therefore they have been positively selected in some populations.[10] The haplotype HLA A28*B39*DR4 is from autochthonous origin (Ameridian) whereas the haplotype HLA A1*B8*DR3 is characteristic of Caucasian populations.[11] There was a large heterogeneity with relatively high frequency of autochthonous (Amerindian) haplotypes with similar lineage patters to those reported for other Amerindian populations.[10]

The mechanism for which extended MHC increase the risk for ATR is probably due to the result of selection for increased immune responsiveness. For instance, extended MHC haplotypes have been associated with autoimmune diseases and this finding is strongly associated with ancestry.[12] Extended MHC haplotypes are related to poor response to hepatitis B immunization and transfusion associated graft *vs.* host disease.[13,14]

Likewise, LD haplotypes DR*3-DQ*2 and DR*4-DQ*3 are associated with type 1 diabetes.[15] In addition, the haplotype A1*B8*DR3, a common haplotype of northern European populations, has been associated with IgA deficiency, common variable immunodeficiency, rheumatoid arthritis, *myasthenia gravis*, dermatitis herpetiform is

amongst other diseases.[16] Alternatively, these haplotypes bear a C4a deletion, which might also have a role in ATR.[17]

There is limited data on the role of extended MHC in the kidney transplant population. Ancestry, in some studies, has been associated with poor allograft survival. Creemers, *et al.*, identified the specific HLA A30*B42*DR3,[17,18] a haplotype of African descent, to be independently associated with poor graft survival.[18]

In a study cohort of 79,000 patients, African-American race was an independent risk factor for graft failure therefore, African-American ancestry has also been associated with poor transplant outcomes.[19] In the Mexican population there is emerging data regarding the role of extended haplotypes in kidney rejection.

In a recent study by Torres-Machorro, *et al.*, the haplotype HLA-HLA-A*02/-B*15 was associated with acute rejection, a null finding in our study.[20] Conversely to our study, Torres-Machorro, *et al.*, evaluated the association of HLA molecules in a pair model in order to use deltas for LD and find the combinations that best predicted rejection. The difference in the results may in part, be due to the fact that Torres-Machorro, *et al.*, included patients from a single institution, while our study included patients from two institutions, thus incorporating patients with different background, making this population more diverse in its ancestry and hereditary lineage patterns. Another difference could be related to the fact that Torres-Machorro, *et al.*, used HLA alleles of the locus A, B and DR as individuals to be able to combine them amongst each other in an arbitrary form, which make it prone to associations with ATR due to chance.

Why are extended MHC associated with acute kidney transplant rejection? The etiology is probably multifactorial, however increased immune responsiveness is one of the potential explanations. Although donor-recipient HLA matching is known to confer allograft survival advantage, our results explore the possible independent association of extended MHC in acute transplant rejection.

The limitations of our study include a relatively small study sample; however with 18 episodes of ATR (36 haplotypes) we had a predictive power of 62.8% to detect a significant difference for the donor's haplotype A1*B8*DR3 and 72% for the recipient's haplotype A28*B39*DR4. Future directions may include DNA sequencing in order to identify non-HLA genes, for example, those within the class III region of the MHC genes such as HSP-70 (Heat Shock proteins), TNF and complement.

In the present study we demonstrate the role of extended MHC in the risk of ATR in the Mexican population. These findings may help direct immunosuppression therapies in these higher risk subjects. More studies with larger number of subjects and longer follow up time are warranted to confirm this association.

REFERENCES

1. Chakraborty R. Gene admixture in human populations: models and predictions. *Yearb Phys Anthropol* 1986; 29: 1-43.
2. Yunis EJ, Larsen CE, Fernandez-Viña M, Awdeh ZL, Romero T, et al. Inheritable variable sizes of DNA stretches in the human MHC: Conserved extended haplotypes and their fragments or blocks. *Tissue Antigens* 2003; 62: 1-20.
3. Brenner S, Miller J (eds.). Encyclopedia of Genetics. Up to Date Glossary of genetic terms. San Diego, CA: Academic Press; 2001.
4. Thorsby E. Invited anniversary review: HLA associated diseases. *Hum Immunol* 1997; 53: 1-11.
5. Yunis EJ, Alper CA, et al. Extended major histocompatibility complex haplotypes in type I diabetes mellitus. *J Clin Invest* 1984; 74: 449-54.
6. McCluskey J, Kay PH, Dawkins RL, Komori K, et al. Association of specific MHC supratypes with rheumatoid arthritis and insulin-dependent diabetes mellitus. *Dis Markers* 1983; 1: 197-212.
7. Sung YCh. The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications. *Yonsei Med J* 2007; 48(1): 11-23.
8. Zhang W, Dolan ME. Ancestry-related differences in gene expression: findings may enhance understanding of health disparities between populations. *Pharmacogenomics* 2008; 9(5): 489-92.
9. Silva-Zolezzi I, Hidalgo-Miranda A, Estrada-Gil J, Fernández-López JC, et al. Analysis of genomic diversity in Mexican Mestizo populations to develop genomic medicine in Mexico. *Proc Natl Acad Sci USA* 2009; 106(21): 8611-16.
10. Barquera R, Zúñiga, Granados J, et al. HLA class I and class II haplotypes in admixed families from several regions in Mexico. *Mol Immunol* 2008; 45(4): 1171-8.
11. Aly T, Eller E, Ide A, Gowan K, et al. Multi-SNP analysis of MHC region remarkable conservation of HLA-A1-B8-DR3. *Haplot Diab* 2006; 55: 1265-9.
12. Fraser PA, Yunis EJ, Alper CA. Excess admixture proportion of extended major histocompatability complex haplotypes of Caucasian origin among rheumatoid arthritis associated haplotypes in African Americans and Afro-Caribbeans. 1996; 1(2): 153-9.
13. Kruskall MS, Alper CA, Awdeh Z, Yunis EJ, et al. The immune response to hepatitis B vaccine in humans: inheritance patterns in families. *J Exp Med* 1992; 175(2): 495-502.
14. Remberger M, Persson U, Hauzenberger D, Ringden O. An association between human leucocyte antigen alleles and acute and chronic graft-versus-host disease after allogeneic haematopoietic stem cell transplantation. *Br J Haematol* 2002; 119(3): 751-9.
15. Noble JA, Valdes AM, Cook M, Klitz W, et al. The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. *Am J Hum Genet* 1996; 59: 1134-48.

16. Price P, Witt CS, Allcock RJN, et al. The genetic basis for the association of the 8.1 ancestral haplotype (A1,B8, DR3) with multiple immunopathological diseases. *Immunol* 1999; 167: 257-74.
17. Candore G, Modica M, Lio Do, et al. Pathogenesis of autoimmune diseases associated with 8.1 ancestral haplotype: a genetically determined defect of C4 influences immunological parameters of healthy carriers of the haplotype. Biomedicine & pharmacotherapy = Biomédecine & pharmacotherapy 2003; 57(7): 274-7.
18. Creemers PC, Kahn D. A unique African HLA haplotype may identify a population at increased risk for kidney graft rejection. *Transplantation* 1998; 65(2): 285-7.
19. Chakkera HA, O'Hare AM, Johansen KL, Hynes D, et al. Influence of race on kidney transplant outcomes within and outside the Department of Veterans Affairs. *J Am Soc Nephrol* 2005; 16(1): 269-77.
20. Torres-Machorro A, Camorlinga-Tagle N, Rodríguez-Ortiz C, Palafox D, et al. Role of major histocompatibility complex and ethnicity in acute renal graft rejection. *Transplant Proc* 2010; 42(6): 2372-5.

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