The association of major histocompatibility complex genes and susceptibility to systemic lupus erythematosus among the Malaysian Chinese population

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Abstract

To determine whether the HLA class II genes, HLA-DR, DQ and DP, carry increased risk to the development of systemic lupus erythematosus (SLE), we studied the frequency of HLA-DR antigens and HLA-DQA and DQB, and DPB alleles among 70 Malaysian Chinese patients with SLE and compared them to 66 ethnically-matched healthy controls by the modified PCR-RFLP technique. The HLA-DR antigens did not show any significant association with SLE. However, a significantly increased frequency of HLA-DQA1*0102 was found among the patients (43.6 vs. 33.3%, $p = 0.004$) and even after correction showed significant result (p corr = 0.03, RR = 3.39). HLA-DQB1*0501 and *0601 were found increased among the patients and remained significant even after correction was made (RR = 4.55 and 4.22 respectively). HLA-DPB1*0901 was also significantly associated with the disease, with a relative risk of 4.58. Our findings suggest that the DQA1*0102, DQB1*0501, *0601 and HLA-DPB1*0901 genes may contribute towards determining SLE disease susceptibility among the Malaysian Chinese population.

Key words: systemic lupus erythematosus, HLA, susceptibility, autoimmunity, Chinese, Malaysia

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease. Although the aetiology is thought to be multifactorial, genetic factors have been known to play a role in disease pathogenesis. Genetic susceptibility to SLE is suggested by population and twin studies, where there is an increased concordance of SLE among monozygotic versus dizygotic twins (Deapen *et al.*, 1992; Block *et al.*, 1975). Genes within the major histocompatibility complex (MHC) have been demonstrated to contribute to SLE. Several investigators have demonstrated that the frequency of HLA-B8, DR2 and DR3 with highly associated DQ antigens to be increased in Caucasians patients with SLE (Hartung *et al.*, 1989; Fronek *et al.*, 1990) while among the American blacks, association was found with DR3, (Alarif *et al.*, 1982), both DR2 and DR3 (Kachru *et al.*, 1984) and DR7. However, these have not been shown in other studies (Howard *et al.*, 1986; Olsen *et al.*, 1989, Reveille *et al.*, 1989).

SLE susceptibility studies among the South East Asians have demonstrated a strong association with DR2 (Hong *et al.*, 1994; Hawkins *et al.*, 1987; Doherty *et al.*, 1992) but this was not found among the Singaporean Chinese (Savage *et al.*, 1995). No association was found with DR3. Associations with the DQA1 gene have also been observed (Lu *et al.*, 1997). There is as yet no study to demonstrate the role of the HLA-DPB locus in conferring increased risk to the development of SLE among the Chinese population of Malaysia. In this study we determined the frequencies of HLA-DR, DQA, DQB and DPB alleles among the Malaysian Chinese SLE patients and investigated their association with SLE susceptibility.

Materials & Methods

Seventy Chinese SLE patients who were on follow-up at the Outpatient SLE Clinic of the National University Hospital of Malaysia were included in the study. All of them fulfilled four or more of the revised criteria of the American College of Rheumatology (formerly American Rheumatism Association) for the classification of SLE (Tan *et al.*, 1982). There were 64 (91%) females and 6 (9%) males giving a female to male sex ratio of 10.6:1. The mean age at study entry was 33 ± 12 years (mean ± sd) with ages ranging from 15-69 years. The mean age at disease onset was 25 ± 10 years (range, 10-58 years) while the mean disease duration was 8 ± 6 years. Sixty-six randomly selected blood donors with no history of rheumatic disease and ethnically-matched served as controls.

HLA typing

Genomic DNA was purified from peripheral blood leucocytes using the salting-out method (Miller *et al.*, 1988). DNA typing for "broad" DR groups (DR1, 2, 3, 4, 5, 6, 7, 8, 9, 10) were determined by PCR while DQA1, DQB1 and DPB1 genotyping was performed by a modified PCR-RFLP (Ota *et al.*, 1991; Nomura *et al.*, 1991). Genomic DNA was amplified by the PCR procedure with 2.5 units of the Taq DNA polymerase (Fermentas AB, Lithuania). The reaction mixture (100µl) containing dNTPs (200 µM) and 2.5 mM MgCl was subjected to 35 cycles of 1 min at 96°C, 1 min at 55°C and 2 min at 72°C by an automated PCR thermocycler (Perkin Elmer Cetus Inc) for the DQB1 gene. As for the DQA1 and DPB1 gene, the reaction mixture was subjected to 30 cycles of 1 min at 94°C, 1 min at 62°C and 2 min at 72°C. After amplification, aliquots of the reaction mixture were digested by restriction endonucleases at optimum temperature after adding incubation buffer and distilled water. FokI, Apa 1, Hae II, SfaNI and BssHII, HphI, BglI and Sac 1. AcyI and HpaII were used for digestion of the amplified DQB1 gene, ApaLI, HphI, BsaJI, FokI, MboII and MnlI for DQA1 and Bsp12861, FokI, DdeI, BsaJI, BssHII, Sau96I, RsaI, EcoNI and AvaII for the amplified DPB1 gene. Electrophoresis was then performed and cleavage or no cleavage was detected by staining with ethidium bromide. Alleles were assigned by comparing RFLP patterns obtained with the relevant charts given.

Statistical analysis

HLA class II antigen and allele frequencies in SLE patients and controls were done using the chi-square analysis with Yates correction of 2 x 2 table (EPI-INFO statistical program, CDC, Atlanta, GA). A p value of <0.05 was taken to be significant. All significant values were multiplied with the number of comparisons made (p corr). The cross product ratio was used to calculate the odds ratio defining the strength of the association between a risk factor and the disease.

Results

HLADR antigen frequency

Table 1 shows the frequencies of the broad HLA-DR antigens in Chinese SLE patients and ethnically matched controls obtained from the Blood Bank of Kuala Lumpur Hospital. Overall, we did not find any significant association of the HLA-DR gene with SLE. However, HLA-DR5 and HLA-DR10 were found to be slightly decreased among the patient group compared to controls (22.9 vs. 45.5%, p = 0.005 and 12.1 vs. 1.4%, p = 0.02 respectively). However, this did not remain significant after correction for the number of alleles tested (p corr = 0.05 and p corr = 0.2 respectively).

HLADQA1 and HLADQB1 allele frequency

Table 2 shows the HLA-DQA1 and DQB1 allele frequencies in SLE patients and controls. Here, we found an increased frequency of HLA-DQA1*0102 (61 of 70, 43.6%) as compared to controls (44 of 66, 33.3%) (p = 0.004, RR = 3.39) which when corrected still remained significant (p corr = 0.032). The other DQA1 alleles did not show any significant differences between patient group and controls. As for HLA-DQB1 alleles, only *0501 and *0601 showed significant differences in the patient group even after correction, (27.1 vs. 7.6%, p = 0.0027, p corr = 0.003, RR = 4.55 and 40 vs. 13.6%, p = 0.0005, p corr = 0.006, RR = 4.22, respectively). Although the frequencies of HLA-DQB1*0502, *0301, *0302, and *0401 were found to be slightly decreased in the patient group (p = 0.035, 0.037, 0.017 and 0.02 respectively), the differences were not

Table 1. Frequency of HLA-DR antigens in Chinese SLE patients and healthy ethnically matched controls

DR	SLE patients n = 70 (%)	Controls n = 66 (%)	p value	p corr	RR
1	1 (1.4)	1 (1.5)	ns		0.94
2	58 (82.9)	50 (75.8)	ns		1.55
3	13 (18.6)	12 (18.2)	ns		1.03
4	13 (18.6)	9 (13.6)	ns		1.44
5	16 (22.9)	30 (45.5)	0.0053	0.053	0.36
6	8 (11.4)	12 (18.2)	ns		0.58
7	6 (8.6)	1 (1.5)	ns		6.09
8	5 (7.1)	6 (9.1)	ns		0.77
9	6 (8.6)	5 (7.6)	ns		1.14
10	1 (1.4)	8 (12.1)	0.02	0.2	0.11

ns = not significant; p corr = p corrected; RR = relative risk

Table 2. Frequencies of HLA-DQA1 and DQB1 alleles in Chinese patients with SLE and controls

	SLE patients n = 70 (%)	Controls n = 66 (%)	p value	p = corr	RR
DQA1					
0102	61 (43.6)	44 (33.3)	0.004	0.032*	3.39
0601	5 (7.1)	10 (15.2)	ns		0.43
0501	22 (31.4)	27 (40.9)	ns		0.66
0301	33 (47.1)	27 (40.9)	ns		1.29
0103	8 (11.4)	5 (7.6)	ns		1.57
0201	7 (10)	10 (15.2)	ns		0.62
0401	2 (2.9)	3 (4.5)	ns		0.62
0101	2 (2.9)	4 (6.1)	ns		0.46
DQB1					
0501	19 (27.1)	5 (7.6)	0.0027	0.003*	4.55
0502	9 (12.9)	18 (27.3)	0.035	0.42	0.39
0503	13 (18.6)	11 (16.7)	ns		1.14
0601	28 (40)	9 (13.6)	0.0005	0.006*	4.22
0602	6 (8.6)	3 (4.5)	ns		1.97
0604	5 (7.1)	4 (6.1)	ns		1.19
0301	11 (15.7)	21 (31.8)	0.027	0.32	0.4
0302	24 (34.3)	36 (54.5)	0.017	0.2	0.43
0303	2 (2.9)	6 (9.1)	ns		0.29
0201	23 (32.9)	12 (18.2)	0.005	0.06	2.2
0401	0 (0)	5 (7.6)	0.02	0.24	0
0402	0 (0)	2 (3)	ns		0

*significant; ns = not significant; p corr = p corrected; RR = relative risk

Table 3. HLA DPB1 allele frequencies in Chinese SLE patients and healthy controls

DPB1	SLE patients n = 70 (%)	Controls n = 66 (%)	p value	p = corr	RR
0101	14 (20.0)	10 (15.2)	ns		1.40
0201	7 (10.0)	12 (18.2)	ns		0.50
0202	0 (0.0)	0 (0.0)	ns		0.00
0301	5 (7.1)	4 (6.1)	ns		1.19
0401	27 (38.6)	19 (28.8)	ns		1.55
0402	12 (17.1)	14 (21.2)	ns		0.77
0501	2 (2.9)	4 (6.1)	ns		0.46
0601	0 (0.0)	0 (0.0)	ns		0.00
0801	2 (2.9)	10 (15.2)	ns		0.16
0901	22 (31.4)	6 (9.1)	0.0012	0.02*	4.58
1001	6 (8.6)	6 (9.1)	ns		0.94
1101	0 (0.0)	0 (0.0)	ns		0.00
1301	10 (14.3)	2 (3.0)	0.04	0.76	5.33
1401	7 (10.0)	6 (9.1)	ns		1.11
1501	0 (0.0)	0 (0.0)	ns		0.00
1601	1 (1.4)	2 (3.0)	ns		0.46
1701	1 (1.4)	3 (4.5)	ns		1.30
1801	1 (1.4)	0 (0.0)	ns		0.00
1901	0 (0.0)	2 (3.0)	ns		0.00

*significant; ns = not significant; p corr = p corrected; RR = relative risk

statistically significant. As for allele *0201, it was slightly increased among the patients (32.9 vs. 18.2%, p = 0.05) as compared to controls, but statistically not significant.

HLADPB1 allele frequency

As shown in Table 3, only *0901 showed significant increase among the patients as compared to controls, even after correction (31.4 vs. 9.1%, p corr = 0.02, RR = 4.58). As for *0801, the frequency among the patients was slightly decreased though not significantly (2.9 vs. 15.2%). However, the frequency of *1301 was slightly increased in the patient group (14.3 vs. 3%) but this too, was not found to be significant after correction. The frequencies of the other HLA-DPB1 alleles were not significantly different between the two groups.

Discussion

Previous studies have demonstrated significant increase of certain components of the MHC among patients with SLE while others show no association of these genes with disease susceptibility. The role of the MHC in determining increased risk to SLE has not been widely studied among the Malaysian Chinese population. Doherty *et al.* (1992), using RFLP found HLA-DRw15 (subtype of DR2) and DQw1 genes to be significantly more frequent among the Malaysian Chinese SLE patients. However, the role of the HLA-DPB1 alleles in SLE susceptibility was not investigated. In this study, besides examining the frequencies of HLA class II DR antigens, DQA1, DQB1 alleles, we have also investigated the role of the DPB1 alleles among 70 Chinese SLE patients in Malaysia and compared them to ethnically-matched controls to ascertain whether these genes play a role in disease susceptibility. We found that there was no significant association of the HLA-DR gene with SLE. However, we noticed a weak association with DR5 and DR10 (decreased among the patient group) but this was not significant when p was corrected for the number of comparisons made. In contrast Hawkins *et al.* (1987) and Hong *et al.* (1994) observed a strong association of SLE with DR2 among their Chinese population. However this finding was not seen among the Singaporean Chinese (Savage *et al.*, 1995). Lu *et al.* (1997), in his study of Taiwanese SLE patients noticed a weakly increased frequency of DR2. Hirose *et al.* (1988) found the frequency of DR2 to be significantly increased in 85 Japanese patients with SLE but the p corrected value did not show significance. In addition, a negative association was seen between SLE and DR5 and DRw6 when compared to his second group of controls (data from 460 individuals of the Third Asia Oceania Histocompatibility Workshop). The association with DR3 as observed in the Caucasian population (Arnett & Reveille, 1992; Schur, 1995; Reveille *et al.*, 1995b; Goldstein & Sengar, 1993; Scherak *et al.*, 1979; Reinharz *et al.*, 1991; Cowland *et al.*, 1994; Skarsvag *et al.*, 1992) was not seen among the Chinese population where this gene occurs quite rarely. Others have not found any HLA-DR association with SLE (Reveille *et al.*, 1995a; Marintchev *et al.*, 1995). The frequency of DR2 and DR3 among our cohort was similar between patients and controls (82.9 vs. 75.8% and 18.6 vs. 18.2% respectively). This could probably be due to the clinical heterogeneity of the patients studied and may also reflect the effects of ethnicity.

HLA-DQA and DQB associations with SLE have been widely studied. In our study, we found a strong association of HLADQA1*0102, DQB1*0501 and *0601 with SLE. Doherty *et al.* (1992) found a weak association with DQw1 and Lu *et al.* (1997) found a weak association with DQB1*0501 and *0602 which did not remain significant after correction was made for multiple comparisons of alleles. However, Cowland *et al.* (1994) and Skarsvag *et al.* (1992) reported an increased frequency of DQA1*0501 and DQB1*0201 haplotype in Danish and Norwegian patients with SLE respectively. In contrast Marintchev *et al.* (1995) who studied Bulgarian SLE patients noticed no significant increase of the HLA-DQA1 or DQB1 genes, and this was in agreement with the findings of Reveille *et al.* (1995a) in their study of Greek SLE patients. However in another study by Reveille *et al.* (1995b), there was no increase in the frequency of DQA alleles but DQB1*0201 and *0602 were slightly more frequent in patients but this was not statistically significant.

Very few studies have investigated the role of the DPB locus in conferring disease susceptibility. While some have found positive associations (Yao *et al.*, 1993; Galeazzi *et al.*, 1992), others have not (Reveille *et al.*, 1995a; Reveille *et al.*, 1992; Marintchev *et al.*, 1995; Davies *et al.*, 1993). In our study, we found a strong association of the allele*0901 with SLE among the Chinese. The strong association of HLA-DQA1*0102, DQB1*0501 and *0601 and HLA-DPB1*0901 with SLE among our study cohort shows the association between SLE and HLA genetic markers, suggesting the role of the MHC genes in pathogenesis of the disease.

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References

Alarif LI, Ruppert GB, Wilson R & Barth WF (1982). HLA-DR antigens in blacks with rheumatoid arthritis and systemic lupus erythematosus. *Journal of Rheumatology* 10, 297-300.

Arnett FC & Reveill JD (1992). Genetics of SLE. *Rheumatic Disease Clinics of North America* 865-892.

Block SR, Winfield JB & Lockshin M (1975). Studies of twins with systemic lupus erythematosus: a review of the literature and presentation of 12 additional sets. *American Journal of Medicine* 59, 533-552.

Cowland JB, Andersen V, Halberg P & Morling N (1994). DNA polymorphism of HLA class II genes in systemic lupus erythematosus. *Tissue Antigens* 43, 34-37.

Davies EJ, Hillarby MC, Cooper RG, Hay EM, Green JR, Shah S, Bernstien RM, Holt PJ & Grennan DM (1993). HLA-DQ, DR and complement C4 variants in SLE. *British Journal of Rheumatology* 32, 870-875.

Deapen DM, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P, Walker A & Mack TM (1992). A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis and Rheumatism* 35, 311-318.

Doherty DG, Ireland RO, Demaine AG, Wang F, Veerapan K, Welsh KR & Vergani D (1992). Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus in Southern Chinese. *Arthritis and Rheumatism* 35, 641-646.

Fronek Z, Timmerman LA, Alper CA, Hahn BH, Kalunian K, Pterelin BM & McDevitt HO (1990). Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus. *Arthritis and Rheumatism* 33, 1542-1553.

Galeazzi M, Sebastiani GD, Passiu G, Angelini G, Delfino L, Asherson RA, Khamashta MA & Hughes GR (1992). HLA-DP genotyping in patients with systemic lupus erythematosus: correlations with autoantibody subsets. *Journal of Rheumatology* 19, 42-46.

Goldstein R & Sengar DP (1993). Comparative studies of the major histocompatibility complex in French Canadian and non-French Canadian Caucasians with systemic lupus erythematosus. *Arthritis and Rheumatism* 36, 1121-1127.

Hartung K, Fontana A, Klar M, Krippner h, Jorgens K, Lang B, Peter HH, Pichler WJ, Schendel D, Robin-Winn M, et al. (1989). Association of class I, II and III MHC gene products with SLE- results of a Central European Multicenter study. *Rheumatology International* 9, 13-18.

Hawkins BR, Wong KL, Wong RWS, Chan K H, Dunkley H & Serjeantson SW (1987). Strong association between the major histocompatibility complex and systemic lupus erythematosus in Southern Chinese. *Journal of Rheumatology* 14, 1128-1131.

Hirose S, Ogawa S, Nishimura H, Hashimoto H & Shirai T (1988). Association of HLA-DR2/DR4 heterozygosity with SLE in Japanese patients. *Journal of Rheumatology* 15, 1489-1492.

Hong GH, Kim HY, Takeuchi F, Nakano K, Yamada J, Matsuta K, Hahn K, Tokunaga K, Ito K & Park KS (1994). Association of complement C4 and HLADR alleles with systemic lupus erythematosus in Koreans. *Journal of Rheumatology* 21, 442-447.

Howard PF, Hochberg MC, Bias WB, Arnett FC & Mclear RH (1986). Relationship between C4 null genes, HLA-D region antigens, and genetic susceptibility to systemic lupus erythematosus in Caucasian and Black Americans. *American Journal of Medicine* 81, 187-193.

Kaehru RB, Sequeira W, Mittal KK, Siegel ME & Telischi M (1984). A significant increase of HLA-DR3 and DR2 in systemic lupus erythematosus among blacks. *Journal of Rheumatology* 11, 471-474.

Lu LY, Ding WZ, Fici D, Deulofeut R, Cheng HH, Cheu CC, Sung PK, Schur PH & Fraser PA (1997). Molecular analysis of major histocompatibility complex allelic association with SLE in Taiwan. *Arthritis and Rheumatism* 40, 1138-1145.

Marintchev LM, Naumova EJ, Rashkov RK, Arnett FC & Reveille JD (1995). HLA class II alleles and autoantibodies in Bulgarians with SLE. *Tissue Antigens* 46, 422-425.

Miller SA, Dykes DD, Polesky HF (1988). A simple salting out proeedure for extracting DNA from human nucleated eells. *Nucleic Acid Research* 85, 1215.

Nomura N, Ota M, Tsuji K & Inoko H (1991). HLA-DQB1 genotyping by a modified PCR-RFLP method combined with group-specific primers. *Tissue antigens* 38, 53-39.

Olsen ML, Goldstein R, Arnett FC, Duvic M, Pollack M & Reveille JD (1989). C4 gene deletion and HLA associations in black Americans with systemic lupus erythematosus. *Immunogenetics* 30, 27-33.

Ota M, Seki T, Nomura N, Sugimura K, Mizuki N, Fukushima H, Tsuji K & Inoko H (1991). Modified PCR-RFLP method for HLA-DPB1 and DQA1 genotyping. *Tissue Antigens* 38, 60-7116.

Reinbarz D, Tiercy JM, Mach B & Jeannet M (1991). Absence of DRw15/7 heterozygotes in Caucasian patients with systemic lupus erythematosus. *Tissue Antigens* 37, 10-15.

Reveille JD, Arnett FC & Olsen ML (1995a). HLA-class II alleles and C4 null genes in Greeks with SLE. *Tissue Antigens* 46, 417-421.

Reveille JD, Brady J, MacLeof-St, Clair M & Durban E (1992). HLA-DPB1 alleles and autoantibody subsets in systemic lupus erythematosus, Sjogrens syndrome and progressive systemic sclerosis: a question of disease relevance. *Tissue Antigens* 40, 45-48.

Reveille JD, Moulds JM & Arnett FC (1995b). Major histocompatibility complex class II and C4 alleles in Mexican Americans with SLE. *Tissue Antigens* 45, 91-97.

Reveille JD, Schrohenloher RT, Acton RT & Barger BO (1989). DNA analysis of HLA-DR and DQ genes in American blacks with systemic lupus erythematosus. *Arthritis Rheum* 32, 1243-1251.

Savage DA, Ng SC, Howe HS, Ngai JLF & Hui KM (1995). HLA and TAP associations in Chinese SLE. *Tissue Antigens* 46, 213-216.

Scherak O, Smolen JS & Mayr WR (1979). Prevalence of HLA-DRw2 not increased in systemic lupus erythematosus (letter). *New England Journal of Medicine* 301-612.

Schur PH (1995). Genetic of systemic lupus erythematosus. *Lupus* 4, 425-37.

Skarsvag S, Hansen KE, Hoist A & Moen T (1992). Distribution of HLA class II alleles among Scandinavian patients with systemic lupus erythematosus (SLE): an increased risk of SLE among non DRB1* 03,DOA1* 0501, DOB1 *0201 class II homozygotes. *Tissue Antigens* 40,128-133.

Tan EM, Cohen AS, Fries JF, Masi AT, McShane D, Rothfield NF, Schaller J, Talal N & Winchester R (1982). The 1982 revised criteria for the classification of SLE. *Arthritis and Rheumatism* 25,1271-7

Yao Z, Hartung K, Deicher HG, Brunnler G, Bettinotti MP, Keller E, Paul C, Gawron C, Mikschl S, Albert E and members of the SLE study group (1993). DNA typing for HLA-DPB1 allele in German patients with systemic lupus erythematosus using the polymerase chain reaction and DIG-ddUTP labelled oligonucleotide probes. *European Journal of Immunogenetics* 20, 259-266.