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CONCISE REPORTS

Predominance of HLA-DRB1*0405 in Korean patients with rheumatoid arthritis

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Abstract

***Objective*—To identify the association of HLA-DR4 subtypes with rheumatoid arthritis (RA) in Koreans.**

***Methods*—Ninety five patients with RA and 118 normal control subjects were examined for HLA-DR antigens by serology. Subtypes of HLA-DR4 were determined by allele specific oligonucleotide typing.**

***Results*—The phenotype frequency of HLA-DR4 in RA patients was significantly greater than that in controls (60·0% versus 31·4%, odds ratio (OR) 3·28, 95% confidence interval (CI) 1·79 to 6·02 ($p < 0·001$)), but HLA-DR6 was decreased in RA patients (15·8% versus 32·2%, OR 0·39, 95% CI 0·19 to 0·81 ($p < 0·001$)). When DR4 was excluded from analysis of patients and controls, the allele frequency of DR1 was significantly increased in the patients compared with controls (11·3% versus 4·5%, OR 2·73, 95% CI 0·87 to 5·95 ($p < 0·001$)). Forty two of 57 DR4 positive patients (73·7%) possessed DRB1*0405, which was strongly associated with RA (44·2% of patients, versus 11·9% of controls: OR 5·88, 95% CI 2·81 to 12·47 ($p < 0·001$)). DRB1*0403 was not found in the patients, but was present in 8·5% of controls. Examining the third hypervariable region at position 70–74 in the DRB1*04 chain by oligotyping, we found that 52 of 57 DR4 positive patients (91·2%) carried one of the conserved amino acid sequences QRRAA or QKRAA, known to be the epitope conferring predisposition to RA.**

***Conclusion*—This study confirms that RA is strongly associated with DR4, especially with DRB1*0405, and that the presence of the inferred QRRAA sequence may be important in susceptibility to RA in Koreans.**

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HLA-DR4 is associated with the development of rheumatoid arthritis (RA) in numerous different populations.[1-9] Recently, it has become possible to study the sequence polymorphism of HLA-DRB1 genes by means of allele specific oligotyping. Nineteen different allelic variants of HLA-DR4 have been found, some of which are associated with RA in different ethnic groups.[4-9] For example, DRB1*0404 and DRB1*0405 were associated with southern Chinese from Hong Kong[7] and Chinese from Shanghai,[6] while DRB1*0405 has been reported to be associated with Japanese[8] and Singaporean Chinese.[9]

A small sequence of nucleotides within the DRB1 gene has been recognised as the epitope conferring predisposition to RA. The amino acid sequences implicated are shared in residues 70–74 of different DRB1 genes, including *0401 (Dw4), *0404 (Dw14), *0405 (Dw15), *0101 (Dw1), and *1402 (Dw16)—a concept known as the 'shared epitope hypothesis'.[10 11]

Korea is a peninsula located between China and Japan. Historically, Korean populations have interacted with Chinese and Japanese, but little is known about the HLA association with RA. We have examined the association of RA with HLA-DR4 subtypes and the susceptibility sequence in the Korean population.

Patients and methods

PATIENTS

Ninety five Korean patients with RA (88 women and seven men; age range 20–62 years) were selected for the study. They were receiving medical care at the Rheumatism Centre in Kangnam St Mary's Hospital and had RA as defined by the American College of Rheumatology.[12] The mean age of onset of RA among the group was 36·2 years, and the mean duration of their disease was 8·3 years (range 1·8–14·7). Of the 95 patients, 75 (78·9%) were rheumatoid factor positive, 42 (44·2%) had extra-articular involvements such as nodules, anaemia, and vasculitis, and 70 (73·7%) had erosive changes in the wrists and fingers. A control group comprised 118 healthy Korean medical students and staff members.

METHODS

HLA-DR serotyping

The microlymphocytotoxicity technique was used.[13] The sera used were well defined ones that had been distributed by the 11th International Histocompatibility Workshop and Conference (11th IHWC).[14]

Genotyping for HLA-DR4 subtypes

The second exons encoding for the first polymorphic domains of the HLA-DRB1*04 gene were selectively amplified by polymerase chain reaction using specific DNA

Table 1 Oligonucleotide sequences of the allele specific probes for HLA-DR4 subtypes

Name	*Amino acid site*	*5'-sequences-3'*	*Specificity against DRB1*04 alleles*
R04	9–13	GAGCAGGTTAAACATGAG	DRB1*04 common
R08	69–74	GAAGACAGGCGGGCCCTG	0412
R14	66–71	GACATCCTGGAAGACGAG	0402
R15	57–62	AGCGCCGAGTACTGGAAC	0405 + 0409 + 0410 + 0411 + 0412
R17	69–75	AGCAGAGGCGGGCCGCGG	0404 + 0405 + 0408 + 0410
R20	57–62	GATGCCGAGTACTGGAAC	0401 + 0402 + 0403 + 0404 + 0406 + 0407 + 0408
R23	81–86	CACAACTACGGGGTTGGT	0401 + 0405 + 0407 + 0408 + 0409
R24	69–75	GCAGAGGCGGGCCGAGGT	0403 + 0406 + 0407 + 0411
R25	33–38	CACCAAGAGGAGTCCGTG	0406
R26	69–75	GCAGAAGCGGGCCGCGGT	0401 + 0409
R27	81–86	CACAACTACGGGGTTGTG	0402 + 0403 + 0404 + 0406 + 0410 + 0411 + 0412

flanking primers (left primer DR13H-1L = CTTGTAGCAGGTTAAACA; right primer PR1 = CGCTGCACTGTGAAGCTCTC). Thirty five cycles were performed: denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and one minute of extension at 72°C. DNA extraction and dot-blot hybridisation were performed according to the 11th IHWC reference protocol.[15] Table 1 lists the allele specific oligonucleotide probes used.

STATISTICAL ANALYSIS

The differences in phenotype frequencies between patients and controls were analysed with two by two tables, χ^2 and Fisher's exact tests. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using Haldane's modification[16] of the method of Woolf. The relative predispositional effects (RPE)[17] were analysed on the basis of the assumption that 52 ambiguous genotypes (observed in 26 patients and 26 controls) were 'heterozygote/blank'.

Results

Table 2 summarises the phenotype frequencies of HLA-DR antigens. HLA-DR4 was significantly increased in patients compared with controls (OR 3·28, 95% CI 1·79 to 6·02 ($p < 0·001$)). HLA-DR6 was decreased in RA compared with controls (15·8% versus 32·2%, OR 0·39, 95% CI 0·19 to 0·81 ($p < 0·001$)). When DR4 was excluded from the RPE analysis for both patients and controls, the allele frequency of DR1 was significantly increased in patients compared with controls (11·3% versus 4·5%, OR 2·73, 95% CI 0·87 to 5·95 ($p < 0·001$)).

Among the 57 HLA-DR4 positive patients, 42 carried DRB1*0405 (73·7%). The phenotype frequency of the DRB1*0405 allele was more significantly increased in RA patients than in controls (44·2% versus 11·9%, OR 5·88, 95% CI 2·81 to 12·47 ($p<0·001$)). DRB1*0403 was decreased in patients compared with controls (0% versus 8·5%, OR 0·05, 95% CI 0·003 to 0·936 ($p=0·002$)), and the other DR4 subtypes—*0401, *0404, *0406, *0407, *0408 and *0410—were not associated with RA (table 3).

The amino acid sequence QRRAA or QKRAA, known to be the RA predisposing epitope, was relatively frequently present in patients. Fifty two of 57 DR4 positive patients (91·2%) possessed one of these sequences. Forty eight of 57 patients (84·2%) had the amino acid sequence QRRAA on the DRB1*04 allele, shared mainly by DRB1*0405.

Discussion

Anthropologically, it is believed that Koreans originated from Palaeoasiatics of a line different from the Chinese, and migrated to the Korean peninsula through Manchuria, the northern part of China, and then to Japan.

Although the pathogenesis of RA is unclear, the association between HLA-DR and genetic susceptibility to RA is well established in different ethnic groups: DR4 in Caucasian, American black, Chinese, and Japanese patients with RA, and DR1 in Asian Indian and Ashkenazi Jewish patients.[1–3]

In our study, HLA-DR4 was found to be significantly associated with RA in Korean patients. DR1 was also demonstrated to be associated with RA, when DR4 was excluded from the analysis by RPE. In contrast, the

Table 2 Phenotype frequencies of HLA-DR antigens in Korean patients with rheumatoid arthritis (RA)

HLA-DR antigens	*RA patients (n = 95) No (%)*	*Controls (n = 118) No (%)*	*OR*	*p*
1†	15 (15·8)	9 (7·6)		NS
2	13 (13·7)	28 (23·7)		NS
3	0 (0·0)	4 (3·4)		NS
4	57 (60·0)	37 (31·4)	3·28	<0·001
5	13 (13·7)	25 (21·2)		NS
6	15 (15·8)	38 (32·2)	0·39	0·009
7	8 (8·4)	14 (11·9)		NS
8	15 (15·8)	25 (21·2)		NS
9	27 (28·4)	28 (23·7)		NS
10	1 (1·0)	2 (1·7)		NS

OR = Odds ratio; NS = not statistically significant ($p > 0·05$).
†Using the RPE method and with DR4 excluded, there is a significant increase in the DR1 allele: observed 15 of 133 (11·3%); expected 6·01 of 133 (4·5%) (odds ratio 2·73, $p < 0·001$).

*Table 3 The distribution of DRB1*04 subtypes and inferred amino acid sequences at position 70–74 in the third hypervariable region of DR4 positive patients*

	Patients (n = 57) No (%)	*Controls (n = 37) No (%)*
DRB1*04 subtype		
0401	4 (7·0)	3 (8·1)
0403	0 (0)**	10 (27·0)
0404	4 (7·0)	6 (16·2)
0405	42 (73·7)***	14 (37·8)
0406	7 (12·3)	3 (8·1)
0407	1 (1·8)	1 (2·7)
0408	0 (0)	1 (2·7)
0410	3 (5·3)	3 (8·1)
Inferred amino acid sequence		
QKRAA	4 (7·0)	3 (8·1)
QRRAA	48 (84·2)†	24 (64·9)
QRRAA or QKRAA	52 (91·2)	26 (70·3)‡

†One patient had DRB1*0405 and *0410.
‡One control had DRB1*0401 and *0405.
Comparisons between the entire patient group (n = 95) and the entire control group (n = 118)—subtype 0403: 0% of RA versus 8·5% of controls, OR = 0·05, **$p < 0·002$; subtype 0405: 44·2% of RA versus 11·9% of controls, OR = 5·88, ***$p < 0·001$.

phenotype frequency of DR1 was not significantly increased in the patients compared with controls (15·8% versus 7·6% ($p > 0·05$)). The association of DR1 with RA in Koreans requires further elucidation. The DR6 antigen was decreased in these patients. Similar data have been reported for Japanese patients.[8] The genetic and clinical significance of these observations remain unknown.

We found the DRB1*0405 allele to be strongly associated with RA in Koreans. A similar finding was reported in Japanese,[8] Singaporean Chinese,[9] and southern Chinese patients.[6 7] Unlike the findings in southern Chinese, DRB1*0404 was not increased in Korean patients; the reason for this difference could be that, historically, Koreans had contact mainly with the northern part of China. The frequency of DRB1*0403 was significantly decreased in the RA patients, and the other alleles (DRB1*0401, *0404, *0406, and *0408) were not associated with RA.

Although the population size in our study was small, it revealed a genetic distribution of DR4 allelic subtypes in association with RA quite different from that in Caucasians, in whom DRB1*0401 and DRB1*0404 are considered to be the RA susceptible genes. However, in the Korean patients RA was associated with DRB1*0405, which is one of the shared epitope alleles that has been reported.

Our study found that 52 of 57 DR4 positive patients (91·2%) carried one of the disease predisposing epitopes, and 48 of 57 patients (84·2%) possessed the amino acid sequence QRRAA. Furthermore, 42 of 48 patients with this epitope sequence shared the subtype DRB1*0405.

The lack of association with the DRB1*0404 allele which shares the inferred sequence of DRB1*0405 may be a reflection of the low frequency of this allele in the Korean population (four of 95 patients (4·2%), six of 118 controls (5·1%)). Gao *et al*[4] suggested that glycine at position 86 may contribute to the specificity that confers the risk for RA; DRB1*0404 contains a valine at position 86 and its role in RA susceptibility may differ from that of DRB1*0401, *0405, and *0101—all of which have glycine at position 86. However, the number of DRB1*0404 alleles in our data was too small to permit discussion of the significance of position 86 of DRB1 in susceptibility to RA in Koreans.

In summary, our results confirm that the HLA-DR4 allele is significantly associated with RA in Korean patients. The prominent allelic subtype of DR4 is DRB1*0405 and there is an increased frequency of the conserved epitope sequence QRRAA on DRB1 molecules. Our findings suggest that the shared epitope hypothesis may be extended to susceptibility to RA in Koreans, for whom the amino acid sequence QRRAA on DRB1 molecules may accordingly be an important genetic element associated with the disease.

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