HLA DQβ restriction fragment length polymorphism and rheumatoid arthritis

Association between DQw7 and rheumatoid arthritis in DR4-positive subjects

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Summary

Two variants of the HLA-DR4-linked DQw3 allele, namely DQw7 and DQw8, were analysed in patients of mixed ancestry (Cape Coloureds) with rheumatoid arthritis and in healthy individuals from the same population group using a DQβ-specific cDNA probe. The DQw7 allele, identified by 3,4 kb *Hind* III or 3,7 kb and 6,9 kb *Bam* HI DQβ-specific restriction fragments, was expressed in 93% of DR4-positive patients ($N = 15$), compared with 12,5% DR4-positive normal individuals ($N = 8$). This DQ variant showed a highly significant association (relative risk = 98; $P < 0,0001$) with rheumatoid arthritis in this population group and may play a role in their susceptibility to this disease.

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Despite intense investigation, the precise HLA-D region gene(s) responsible for susceptibility to rheumatoid arthritis have not been unequivocally identified.[1] HLA-DR4 is strongly associated with rheumatoid arthritis in many, but not all, racial and ethnic groups.[2] Furthermore, HLA-DQ genes are in strong linkage disequilibrium with HLA-DR, and use of DQ-specific gene probes has led to the observation of significant associations between HLA-DQw3-related specificities and myasthenia gravis,[3] diabetes mellitus[4] and rheumatoid arthritis.[5,6] In addition, gene interaction at the DQ locus in heterozygous patients contributes to the level of auto-antibodies in primary Sjögrens syndrome.[7] These data suggest that the real susceptibility marker for the development of many autoimmune diseases may be identical, or closely linked, to DQβ-encoded molecules. Two DR4-associated DQw3 alleles (DQw7 and DQw8) have been defined by serology and by restriction fragment length polymorphisms (RFLP).[8] A study was undertaken to investigate RFLPs in the DQβ gene in DR4-positive adult seropositive patients of mixed ancestry (Cape Coloureds) with rheumatoid arthritis and in healthy individuals of the same race group.

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Patients and methods

Rheumatoid arthritis patients who attended the rheumatology clinic at Groote Schuur Hospital were typed for HLA antigens using the standard National Institutes for Health (NIH) microlymphocytoxicity technique for class I antigens[9] and the 7th International Workshop Histocompatibility technique for class II antigens.[10]

All patients had classic seropositive rheumatoid arthritis. Of the 35 patients, 20 were Cape Coloured (all women) and 15 of these were positive for DR4. Eight healthy unrelated DR4-positive Cape Coloured individuals were analysed as controls.

DNA was prepared from peripheral blood lymphocytes, essentially as described by Kunkel *et al.*[11] Samples of genomic DNA (15 μg) were digested with 40 units of restriction endonucleases *Bam* HI or *Hind* III for 18 hours at 37°C in buffer conditions as prescribed by the manufacturer (Amersham). The digested DNA samples were then electrophoresed in a 0,9% agarose gel in 40mM tris-acetate, 2mM ethylenediaminetetra-acetic acid (EDTA), pH 7,6 for 18 hours at 35 - 40 volts. As a molecular weight marker λ-DNA digested with *Hind* III plus *Eco* RI was used. Upon completion of electrophoresis, the gels were denatured, neutralised and DNA transferred to nylon membrane (Hybond N, Amersham) by the method of Southern.[12] The membranes were prehybridised at 65°C in 3 × SSC [10 × SSC is 1,5M NaCl, 0,5M Na citrate, pH 7,4]; 0,25% Blotto [Blotto is 1% fat-free milk powder], 0,1% sodium dodecyl sulphate (SDS) for 2 - 4 hours, followed by the addition of 0,5 μg nick-translated DQβ cDNA probe, a nearly full-length G-C-tailed cDNA insert in pUC9 (specific activity = $1,6 \times 10^8$ dpm/μg) at a concentration of 10^7 cpm/ml, and hybridised for at least 18 hours. The membranes were subsequently washed at 65°C in 0,1 × SSC, 0,1% SDS for 1 - 2 hours, followed by autoradiography at −70°C for 1 - 14 days as required (Hyperfilm MP, Amersham).

Statistical analysis. The odds ratio was used as an estimate of relative risk to assess the strength of association. Fisher's exact test was used to calculate two-tailed probabilities (*P*-value).

Results

The hybridisation of DQβ-specific cDNA to *Hind* III or *Bam* HI-digested genomic DNA identified the two DQβ variants, DQw7 and DQw8 (formerly DQw3,1 and DQw3,2) on the HLA DR4 haplotypes. The DQw7 allele was identified by 3,4 kb *Hind* III or 3,7 kb and 6,9 kb *Bam* HI DQβ-specific DNA fragments (Figs 1 and 2).

Genomic DNA from 15 DR4-positive rheumatoid arthritis patients was analysed for the presence of specific DQ alleles. The DQw7 allele was expressed in 92,5% of subjects (Table I).

Genomic DNA from 8 normal subjects positive for DR4 was analysed for the presence of DQw3 variants. Seven subjects expressed the DQw8 allele, identified by the presence of a 12

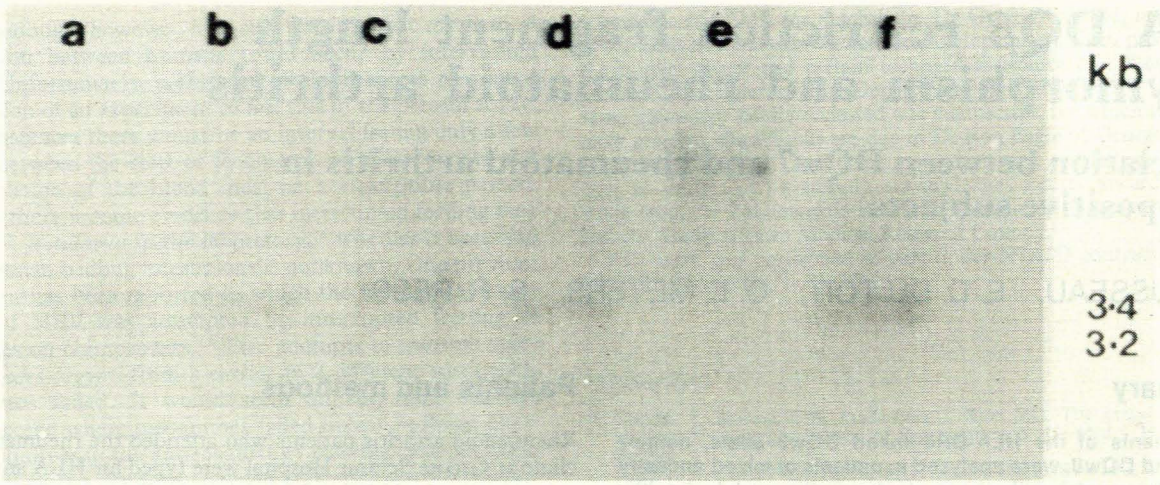

Fig. 1. DNA analysis of DR4 subjects. DNA samples were digested with *Hind* III and probed with DQβ cDNA. Lanes a,c,e and f: DQw7 allele; lanes bd: DQw8 allele.

kb *Bam* HI and a 3,2 kb *Hind* III DQβ-specific restriction fragment. The DQw7 allele was thus present in only 12,5% of healthy DR4 haplotypes.

The relative risk associated with the presence of the DQw7 allele in rheumatoid arthritis patients positive for DR4 was 98 ($P < 0{,}0001$), with 95% confidence limits of 3,8 and 1 359,5.

Discussion

We have demonstrated in this study that the DR4 haplotype associated with adult seropositive rheumatoid arthritis predominantly expresses the DQw7 allele in Cape Coloured patients in whom the incidence of DR4 in rheumatoid arthritis

TABLE I. IDENTIFICATION OF THE DQw7* ALLELE BY 6,9/3,7 KB *BAM* HI AND 3,4 KB *HIND III RFLPs; IDENTIFICATION OF THE DQw8** ALLELE BY 3,2 KB *HIND* III RFLP IN DR4-POSITIVE PATIENTS AND DR4-POSITIVE CONTROLS**

		RFLP		
HLA		*Bam* HI	*Hind* III	
DR	DQ	6,9/3,7 kb*	3,4 kb*	3,2 kb**
Patients				
4,7	w3,w2	ND	+	—
4,w6	w3,w1	ND	+	—
4,5	w3,—	+	+	—
4,—	w3,—	+	ND	ND
3,4	w3,w2	ND	+	—
3,4	w3,w2	ND	+	—
1,4	w3,w1	+	+	—
4,5	w3,—	+	+	—
4,—	w3,—	+	+	—
4,—	w3,—	+	+	—
4,5	w3,w1	+	+	—
4,5	w3,—	ND	+	—
4,—	w3,—	ND	+	—
4,5	w3,—	ND	+	—
2,4	w3,1	—	—	+
Controls				
1,4	w3,w1	—	—	+
3,4	w2,w3	—	—	+
4,—	w3,—	—	—	+
4,5	w3,—	—	—	+
4,—	w3,—	—	—	+
2,4	w3,—	—	—	+
4,8	w3,—	—	—	+
1,4	w3,w1	+	+	—

ND = not done

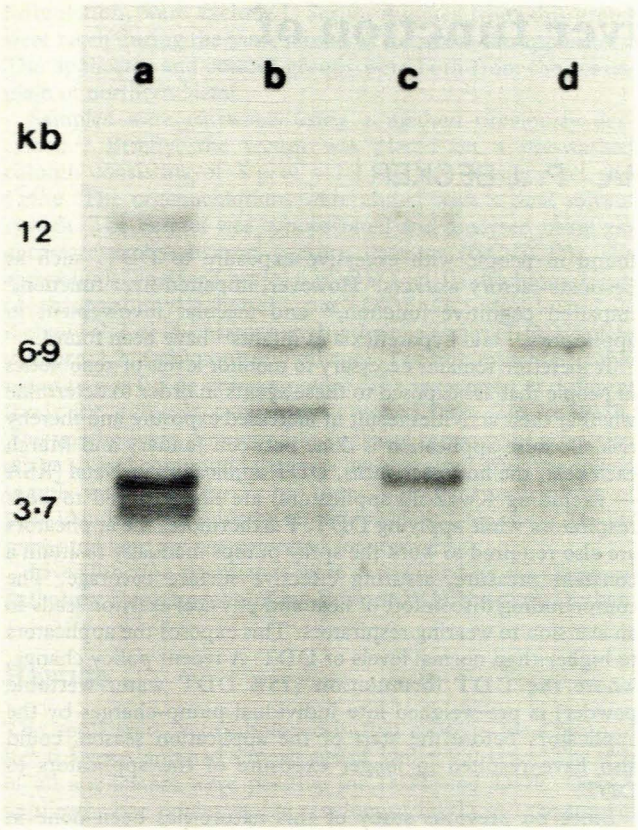

Fig. 2. DNA analysis of DR4 subjects. DNA samples were digested with *Bam* HI and probed with DQβ cDNA. Lanes b,c,d: DQw7 allele; lane a: DQw8 allele.

is 50%.[13] Our results are in agreement with the data of Singal *et al.*,[6] who found that the relative risk associated with the presence of the DQw7 allele in white rheumatoid arthritis patients positive for DR4 was 78. The question therefore arises whether the DQ genomic cluster of alleles are functionally important in immune recognition and the development of rheumatoid arthritis. Evidence for a role for DQ molecules exists in some auto-immune diseases. For example, a 15 kb *Hinc* II DQβ-specific cDNA fragment has been demonstrated in 7/16 DR3-positive patients with myasthenia gravis and in only 1/19 normal subjects.[3] In addition, a 12 kb *Bam* HI DQβ-specific cDNA fragment was shown to be present in 90% DR4-positive IDDM patients.[14]

Alternatively, disease susceptibility may be linked to an associated Dw14 allele as has been detected with the use of oligonucleotide probes.[14]

In conclusion, the results of this small study have demonstrated a clear association between HLA haplotypes and rheumatoid arthritis, with a significant relative risk of 98 for the development of rheumatoid arthritis conferred by the DQw7 variant of DQw3. If confirmed by greater numbers, it may indicate that this HLA-DQβ polymorphism may be identical, or closely linked, to a genetic locus responsible for susceptibility to rheumatoid arthritis.

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