Analysis of T cell receptor V alpha polymorphisms in rheumatoid arthritis

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Abstract

***Objective*—To test for association of T cell receptor (TCR) V alpha polymorphisms and rheumatoid arthritis (RA) in British and Swiss white populations.**

***Methods*—TCRAV polymorphisms were analysed in RA patients and controls by single strand conformational polymorphism (SSCP) analysis. Associations were sought between defined genotypes and RA, and the effect of HLA-DR4 status analysed. Putative associations were then retested further in new groups of patients and controls. Overall, 360 RA patients and 197 controls were studied.**

***Results*—No association between TCRAV5S1, V6S1, V8S1, V17S1 or V21S1 polymorphisms and RA were observed in the initial population screened. Stratification for DR4 status showed an increase of V5S1*01/*01 in DR4 positive versus DR4 negative patients (χ^2 = 7.19, p=0.028 (2df), p=0.14 after correction for multiple comparisons). This putative association was tested in three further patient groups, none of which showed significant increase of V5S1*01/*01 in DR4 positive patients, although an overall trend towards an increase in V5S1*01/*01 was observed.**

***Conclusion*—No evidence was found for a strong association of TCRAV genes and RA in a white population. However, these results suggest a weak association of V5S1*01/*01 with DR4 positive RA, although this requires confirmation using larger groups of patients and controls.**

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Both environmental and genetic factors contribute to the pathogenesis of rheumatoid arthritis (RA). Multiple genes are probably involved in determining susceptibility, the most clearly established being genes on chromosome 6, in the major histocompatibility complex HLA-DR region.[1] Data from family and twin studies have estimated that the HLA component of heritability is approximately 40%, whereas the remaining 60% is caused by as yet unidentified genes.[2] Efforts to identify these other genes can broadly be grouped into three approaches: by looking for linkage with candidate genes, by seeking genetic linkage on a genome wide basis, or by seeking association with candidate genes in a population study.

Using the candidate gene approach, associations between RA and α_1 antitrypsin, immunoglobulin allotypes and polymorphisms of the TCR loci have been reported.[3 4] An association between RA and genes encoded by the TCR loci is conceptually attractive, as the products of TCR genes function as receptors for peptide antigens bound to MHC molecules. The V regions of the TCR molecules interact directly with peptide fragments bound to the MHC, and variations in the V sequence can affect T cell specificity.[5] Although the major source of variation of the TCR repertoire resides in recombinatorial diversity, differences in germ line TCR genes can also influence the repertoire.[6 7]

Recent studies of the TCRA locus have indicated that a number of the V gene subgroups are polymorphic,[8 9] and may be useful markers for disease studies. We have investigated the genetic contribution of TCRAV genes to RA by examining the distribution of five known TCRAV polymorphisms in north European white RA patients and controls using single strand conformational polymorphism (SSCP) analysis. Association between genotype distribution and RA was studied initially in a British group of patients and controls, and subsequently tested in Swiss patients and controls, as well as further UK patients.

Methods

PATIENTS AND CONTROLS

The UK RA patient groups consisted of patients attending rheumatology clinics in South Wales (UKRA1), Hammersmith Hospital, London (UKRA2), and Guy's Hospital, London (UKRA3). Control subjects were healthy hospital volunteers. All British subjects were from a white population.

Swiss RA patients were recruited from the rheumatology clinics of the University hospitals of Geneva and Lausanne. All Swiss patients were of white origin. Swiss controls were consecutive white blood donors.

All patients fulfilled the ACR criteria for the diagnosis of RA.[10] Table 1 shows the characteristics of the patient groups.

SINGLE STRAND CONFORMATIONAL POLYMORPHISM (SSCP) ANALYSIS

SSCPs were performed for TCRAV5S1, V6S1, V8S1, V17S1, and V21S1 polymorphisms as described previously.[9]

DR4 TYPING

Genotyping for DR4 was performed by PCR-SSCP using DR4-specific primers or by a microtitre plate oligotyping assay.[11 12] Table 1 shows the percentage of DR4 positive subjects in RA groups.

Table 1 Characteristics of the RA patient groups

RA patient group	*Number*	*% Female*	*% Seropositive*	*% With erosions*	*% With nodules*	*% HLA-DR4*
UK RA1	75	75	76	96	31	65
UK RA2	66	70	NA	NA	NA	65
UK RA3	87	69	NA	NA	NA	60
Swiss RA	132	74	76	74	30	50

Table 2 Comparison of V5S1 genotypes between DR4+ and DR4− RA patients and controls

HLA-DR4 status		*V5S1*01/*01*	*V5S1*01/*02*	*V5S1*02/*02*	
UK RA 1 DR4+	n=49	31 (0.63)	14 (0.29)	4 (0.08)	χ^2=7.187
UK RA 1 DR4−	n=26	8 (0.31)	14 (0.54)	4 (0.15)	*p=0.0275(2df)
UK RA 2 DR4+	n=43	29 (0.67)	11 (0.26)	3 (0.07)	χ^2=3.9
UK RA 2 DR4−	n=23	10 (0.43)	9 (0.4)	4 (0.17)	p=0.14 (2df)
UK RA 3 DR4+	n=52	28 (0.54)	15 (0.29)	9 (0.17)	
UK RA 3 DR4−	n=35	20 (0.57)	12 (0.34)	3 (0.09)	
Total UK RA DR4+	n=144	88 (0.61)	40 (0.28)	16 (0.11)	χ^2=7.78
Total UK RA DR4−	n=84	38 (0.45)	35 (0.42)	11 (0.13)	p=0.02 (2df)
UK Control DR4+	n=31	15 (0.48)	14 (0.45)	2 (0.07)	
UK Control DR4−	n=78	35 (0.45)	32 (0.41)	11 (0.14)	
Swiss RA DR4+	n=67	37 (0.55)	19 (0.28)	11 (0.16)	
Swiss RA DR4−	n=65	34 (0.52)	26 (0.4)	5 (0.08)	
Swiss Control DR4+	n=19	8 (0.42)	7 (0.37)	4 (0.21)	
Swiss Control DR4−	n=69	47 (0.68)	14 (0.2)	8 (0.12)	
Total RA DR4+	n=211	125 (0.59)	59 (0.28)	27 (0.13)	χ^2=6.63
Total RA DR4−	n=149	72 (0.48)	61 (0.41)	16 (0.11)	p=0.036 (2df)
Total Control DR4+	n=50	23 (0.46)	21 (0.42)	6 (0.12)	
Total Control DR4−	n=147	82 (0.56)	46 (0.31)	19 (0.13)	

*p Value corrected for multiple comparisons=0.0275 × 5=0.14.

STATISTICS

The data were analysed using contingency tables. Odds ratios were calculated as described.[13]

Results

V5S1, V6S1, V8S1, V17S1, and V21S1 genotypes were determined by SSCP on a cohort of British white RA patients and normal subjects (UKRA1). No significant association was found for V5S1, V6S1, V8S1, V17S1, or V21S1 with RA (data not shown).

To investigate the possibility of interaction with HLA-DR4, the control and patient groups were stratified according to DR4 status. No significant differences were observed in the genotype distribution of V6S1, V8S1, V17S1, and V21S1 polymorphisms (data not shown). An increase in V5S1*01/*01 in DR4+ versus DR4− was observed in RA patients but not controls (63% *v* 31% compared with 48% *v* 45% in controls, χ^2=7.19, p=0.028 (2df), p=0.14 after correction for multiple comparisons, see table 2: RA1).

To determine if the increase of the V5S1*01/*01 genotype in relation to HLA-DR4 is reproducible and significant, the genotype distribution of TCRAV5S1 was analysed in three additional groups of RA patients, two from the UK (RA2 and RA3) and one from Switzerland. In the RA2 patient group, a similar increase in the V5S1*01/*01 genotype was seen in DR4+ versus DR4− patients (67% *v* 43%), although this did not reach statistical significance (table 2). The RA3 patient group showed no increase in V5S1*01/*01 in DR4 positive patients (54% *v* 57% in DR4 negative patients, table 2). Pooling of the three UK RA groups showed an overall increase of V5S1*01/*01 in DR4+ *v* DR4− patients (n=228) of 61% *v* 45% (χ^2=7.78, p=0.02 (2df)). The Swiss patient group showed a slight excess of V5S1*01/*01 in DR4+ *v* DR4− patients (55% *v* 52%).

*Table 3 Odds ratios for the association of V5S1*01/*01 with HLA-DR4 positive RA*

RA patient group	*Odds ratio*	*95% Confidence intervals*
RA1	1.8	0.6, 5.2
RA2	2.2	0.7, 6.7
RA3	1.2	0.2, 6.7
UK RA	1.7	0.7, 4.2
Swiss RA	1.7	0.4, 6.8
Total RA	1.7	0.9, 3.4

On pooling the V5S1 data from the UK and Swiss RA patient groups, the *01/*01 genotype was present in 59% of 211 DR4 positive patients, compared with 48% in 149 DR4 negative RA (χ^2 = 6.63, p=0.036 (2df), table 2). No increase of V5S1*01/*01 was seen in the pooled control group, which comprised both UK and Swiss white controls.

Table 3 shows the odds ratios for association of V5S1*01/*01 with HLA-DR4 positive RA. The values for the individual groups ranges from 1.2 for UKRA3 (95% CI 0.2, 6.7) to 2.2 (0.7, 6.7) for UKRA2. The overall odds ratio is estimated at 1.7 (0.9, 3.4; table 3).

Discussion

The inherited basis of RA is likely to be polygenic. To date, the influence of HLA genes is the best studied and has been consistently confirmed in many different populations.[14] TCR genes are candidate susceptibility genes in RA, however analyses of the role of TCR genes in disease have been hampered by suitably polymorphic markers, and the absence of genetic maps of the loci in question. Recently, an association between genes in the TCRBV6S7*1 region of the TCRB complex and RA was reported, which demonstrated an interaction with HLA-DR4.[15] A family study of affected sib pairs also showed suggestion of linkage to genes in the same region.[16] Little is known about the genetic contribution of the TCRA locus to RA. Among other autoimmune diseases, associations between a

TCRA constant region RFLP and systemic lupus erythematosus,[17] and an RFLP of the TCRAV1 subgroup and autoimmune thyroid disease have been reported.[18]

In our initial study, we found no associations between TCRAV polymorphisms and RA overall, but an increase in the V5S1*01/*01 genotype in DR4+ subjects was observed. This prompted us to enlarge our study to include RA patients from other centres in the UK and also from Switzerland. Our initial results could not be significantly reproduced, although two of three further patient groups showed an increase of V5S1*01/*01 in DR4+ subjects. The non-reproducibility of the results may be because of disease heterogeneity between the patient groups studied. However, we believe this is unlikely as all groups are similar in terms of DR4 positivity, sex, and all were recruited from the general population. In addition, from the available clinical data, there seems to be little difference between the characteristics of the UKRA1 group and the Swiss RA patient group, which showed differences in V5S1 genotype distribution (see table 1). Distinct HLA-DRB1 subtypes are associated with RA in white subjects, of which HLA-DRB1*0401 and *0404 show the strongest association.[15] We cannot rule out the possibility that V5S1*01/*01 is associated with a particular DR4−subtype, which may be differentially represented in the patient groups.

Using combined data from all groups we estimate the odds ratio for the putative association of V5S1*01/*01 with DR4 positive RA to be 1.7 (95% CI = 0.9, 3.4), with values for individual groups ranging from 0.2 to 2.2 (table 3). These values are consistent with the previous χ^2 analysis (table 2), indicating a weak affect in all of RA patient groups, although the ratios are not statistically significant. Much larger numbers of patients and controls will be required in future studies to definitively prove or disprove the association.

The strongest genetic association in RA remains with the HLA-DR region, with which assocations have been consistently found.[14] To date, no other genetic susceptibility factor for RA approaching the strength of HLA has been discovered, and it is probable that the genes that contribute to the non-HLA component of susceptibility will individually have weak associations with RA. A similar model has been evoked in the genetics of IDDM, for which the contribution of individual non-HLA loci is predicted to be small, and HLA remains the major susceptibility factor.[19]

From our results, we cannot rule out association of other individual TCRAV genes with RA. A recent study has suggested that TCRAV8S1*02 is associated with RA.[20] Our failure to detect this association may be because of the smaller size of our study, clinical disease heterogeneity, or differences in the HLA background between the patient groups.

In conclusion, our results do not support strong association of TCRAV genes with RA, however, the existence of a minor genetic influence is suggested.

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