CONCISE REPORT

Analysis of the insertion/deletion related polymorphism within T cell antigen receptor β variable genes in primary Sjögren's syndrome

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Objective: To analyse T cell receptor β variable (TCRBV) gene polymorphisms (insertion/deletion related polymorphism (IDRP) and BV6S7) in primary Sjögren's syndrome (PSS).

Methods: Genomic DNA was extracted from blood samples from patients fulfilling the modified European criteria for PSS (n = 61). Healthy control blood samples were obtained from the Blood Transfusion Service (n = 121). As a disease control group, samples from patients with systemic lupus erythematosus (n = 42) were analysed. BV6S7 was genotyped using an established PCR/RFLP method. The IDRP was determined by comparison of the intensity of PCR product bands from within BV9S2 and an internal control region (BV9S1), to ascertain whether 0, 1, or 2 copies of the insertion were present.

Results: There was a decrease ($p=0.018$) in the proportion of PSS patients with the deleted/deleted genotype. There was no association with specific BV6S7 alleles or genotypes with either the PSS group or the hypergammaglobulinaemic subgroup. There were no significant differences in haplotype frequencies after Bonferroni correction.

Conclusions: A reduced proportion of patients with PSS have the deleted/deleted genotype. Eighty nine per cent of PSS patients have at least one extra germline copy of BV13S2*1. This may relate to previous observations of increased BV13 specific T cells and mRNA in the salivary glands.

Primary Sjögren's syndrome (PSS) is a systemic autoimmune disease characterised predominantly by dry eyes and dry mouth.^{1,2} These symptoms are thought to result from lymphocytic infiltration of the salivary and lacrimal glands. Immunohistochemical studies have shown that the majority of infiltrating lymphocytes are CD4⁺ $\alpha\beta$ T cells.^{3,4} In addition, although the repertoire of BV transcripts found in lip biopsies from patients with PSS is not restricted, BV2 and BV13 mRNA and T cells have been found to be over-represented in lip biopsies.⁵ It has been postulated that these BV regions may be involved in the pathogenesis of gland destruction.

The T cell antigen receptor (TCR) is essential for recognition of foreign antigens. Certain polymorphisms in the variable gene segments have also been linked with increased susceptibility to particular diseases.⁶ Variability of the antigen receptor is maintained by recombination of variable (V), diversity (D), joining (J), and constant (C) gene segments that span 685 kb on chromosome 7q35.⁷ In all, the TCR β locus contains 65 unique variable gene (BV) segments, 46 of which are functional and several are polymorphic.

We have analysed two polymorphic loci within the TCR β variable genes in PSS, namely BV6S7 and the insertion/

deletion related polymorphism (IDRP). The IDRP is a 21.5 kb region containing an extra copy of BV13S2*1, a unique extra gene BV7S3, and the pseudogene BV9S2.⁸ TCRBV6S7 is a biallelic single nucleotide polymorphism.⁹ A previous study has suggested an association between BV6S7*2 and hypergammaglobulinaemic PSS.¹⁰

METHODS

Patients and controls

The study was approved by the appropriate local research ethics committee. Genomic DNA was extracted from blood samples obtained from 61 white patients from the Yorkshire and Birmingham regions who fulfilled the revised European criteria for PSS.¹¹ Clinical criteria for PSS were confirmed by a combination of clinical assessment and review of the medical notes. Healthy control samples were obtained through the Yorkshire and Birmingham blood transfusion services (n = 121) to ensure geographical matching of cases and controls. Samples were also obtained from 46 patients fulfilling the American College of Rheumatology criteria for systemic lupus erythematosus (SLE) as a disease control group.¹²

PCR reactions

DNA was amplified in a final volume of 30 μ l, using 100 ng of genomic DNA, 1 $\times$ Taq DNA polymerase buffer A (Promega, Madison, Wisconsin, USA), 10 pmol/ μ l of each primer, 133 μ M dNTP (Invitrogen, San Diego, California, USA), MgCl₂ (Promega) at 3.0 mM for BV6S7 and 2 mM for the IDRP, and 1 unit of Taq DNA polymerase (Promega). Reactions were run on a GeneE polymerase chain reaction (PCR) machine (Techne) under mineral oil. Twenty six cycles were carried out for the IDRP and 30 for BV6S7. Annealing temperatures were 58°C for BV6S7 and 62°C for the IDRP. For BV6S7 the following primers were used: forward, 5' GTC ACA GAG AAG GGA AAG G 3'; reverse, 5' CGG CCG AGT CCT CCT GCT G 3'. The 232 base pair (bp) PCR product from BV6S7 was digested using the restriction enzyme BamHI (10 units) and run on a 3% metaphore gel (Flowgen) containing ethidium bromide. Allele 1 contains a BamHI restriction site that is abolished by the nucleotide change in allele 2. Figure 1 shows photographs of the PCR products resolved by gel electrophoresis.

Two simultaneous PCRs were employed to analyse the IDRP, using a modified form of the PCR described by Charmley and Concannon.¹³ One fragment was amplified from within a control region BV9S1 (two copies present in all individuals) giving a 222 bp product, and a second from within BV9S2 giving a 424 bp product (BV9S2 is part of the

Abbreviations: IDRP, insertion/deletion related polymorphism; PSS, primary Sjögren's syndrome; TCR, T cell antigen receptor; TCRBV, T cell receptor β variable

Figure 1 Gel photographs for insertion/deletion related polymorphism (IDRP) and BV6S7 polymerase chain reaction products. bp, base pair.

IDRP so either 0, 1, or 2 copies are present in any individual). The following primers were used: BV9S1 forward, 5' TTG GGA AAC GAC AAG TCC ATT 3'; BV9S2 forward, 5' TCC TCT GCT ATG TGG CCC 3', and BV9 common reverse primer, 5' CAC AGC AGA GTC ACC AAG C 3'. A comparison of band intensities was used to genotype individuals. If the second band was absent, individuals were homozygous for the deletion. If two equal intensity bands were present, they were recorded as homozygous for the insertion. If the second band was approximately half the intensity of the control band, then they were recorded as heterozygous. PCR products were run on a 2% agarose gel (Gibco, Gaithersburg, Maryland, USA) containing ethidium bromide, and band intensities were formally quantified using a phosphorimager (Molecular Imager FX, BioRad).

Genotypes were then recorded for each individual, and haplotypes assigned for BV6S7 and the IDRP. There are three possible haplotypes for the IDRP, as reported by Zhao *et al.*⁸ The inserted haplotype (I) is associated with BV6S7*1, the deleted haplotype D1 is associated with BV6S7*2, and the deleted haplotype D2 is associated with BV6S7*1. Allele, genotype, and haplotype frequencies were then compared between groups using χ^2 analysis. A Bonferroni correction was carried out to allow for multiple analyses.

RESULTS

There was a decrease ($p = 0.018$) in the proportion of PSS patients with the deleted/deleted genotype in PSS compared

with healthy controls, with a corresponding increase in heterozygotes. In the healthy control group 27.3% had the deleted/deleted genotype, whereas among those with PSS the figures were 10.8% and 12.5% for those with raised or normal IgG concentrations, respectively. This gives an odds ratio of 3.0 for the deleted/deleted genotype in PSS compared with healthy controls. There were no significant differences between PSS and controls with regard to BV6S7 alleles or genotypes (table 1). There were no significant differences in haplotype frequencies after Bonferroni adjustment, or between SLE and controls for any of the analyses. Healthy control data were in Hardy–Weinberg equilibrium.

DISCUSSION

The reduced proportion of PSS patients with the deleted/deleted genotype was our most significant finding. Eighty nine per cent of PSS patients had at least one copy of the insertion and therefore at least one extra germline copy of BV13S2*1. This was demonstrated in the PSS group as a whole, rather than in a specific serological subgroup. This result links with previously published data in that biopsies of salivary glands from PSS patients have been found to have an over-representation of BV13 specific T cells and mRNA compared with biopsies from healthy controls.⁵ We have not sought to replicate that work, but our finding that PSS patients are likely to have at least one extra germline copy of this gene segment could help to explain why the over-representation of BV13 occurs at both the mRNA and protein levels.

Table 1 Allele, genotype, and haplotype frequencies for patients with primary Sjögren's syndrome, systemic lupus erythematosus, and controls		PSS (n = 61)	PSS raised IgG subgroup (n = 37)	PSS normal IgG subgroup (n = 24)	SLE (n = 42)	Controls (n = 121)
BV6S7 genotype frequencies	1.1	0.459	0.541	0.333	0.333	0.446
	1.2	0.459	0.378	0.583	0.452	0.430
	2.2	0.082	0.081	0.083	0.214	0.124
BV6S7 allele frequencies	Allele 1	0.689	0.730	0.625	0.560	0.661
	Allele 2	0.311	0.270	0.375	0.440	0.339
IDRP genotype frequencies	II	0.262	0.297	0.208	0.214	0.273
	ID	0.623	0.595	0.667	0.429	0.455
	DD	0.115	0.108	0.125	0.357	0.273
IDRP allele frequencies	Ins	0.574	0.595	0.542	0.429	0.500
	Del	0.426	0.405	0.458	0.571	0.500
Haplotype frequencies	I	0.574	0.595	0.542	0.429	0.50
	D1	0.311	0.270	0.375	0.44	0.339
	D2	0.115	0.135	0.083	0.131	0.161

IDRP, insertion/deletion related polymorphism; PSS, primary Sjögren's syndrome; SLE, systemic lupus erythematosus.

In contrast to the findings of Kay *et al.*¹⁰ we detected no differences in allele or genotype frequency for BV6S7. This may be a result of geographical or racial variation between control groups, or an effect of the differing classification criteria used for PSS. In fact the trends seen in our data were the opposite to those previously published, with a non-significantly higher frequency of BV6S7*1 homozygotes in the hypergammaglobulinaemic subgroup.

It has previously been postulated that BV13 T cells may be involved in the pathogenesis of gland destruction, as discussed above. Our data suggests a possible genetic mechanism for this. Having at least one extra copy of BV13S2*1 as a result of the insertion allele increases the likelihood that there may be an increased proportion of these cells present in individuals with Sjögren's syndrome.

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