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Polymorphism in the 5′ regulatory region of the B-lymphocyte activating factor gene is associated with the Ro/La autoantibody response and serum BAFF levels in primary Sjögren's syndrome

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Objective. To investigate the association between haplotypes in the 5′ regulatory region of the B-lymphocyte activating factor (*BAFF*) gene, disease susceptibility and serum BAFF (s-BAFF) levels in Caucasian primary SS (pSS) patients.
Methods. Case–control study in an established pSS cohort with PCR–RFLP genotyping for four SNPs (-2841 T→C, -2704 T→C, -2701 T→A, -871 C→T), which tag a haplotype block in the 5′ regulatory region of the BAFF gene and s-BAFF determination by ELISA.
Results. s-BAFF levels were elevated in Ro/La-positive pSS patients ($n = 85$, 1770 pg/ml) compared with both Ro/La-negative pSS patients ($n = 27$, 1193 pg/ml) and controls ($n = 59$, 1171 pg/ml), $P < 0.001$. s-BAFF increased with diversification of the anti-Ro/La antibody response, but was not correlated with age, RF or immunoglobulin G levels. There were four common BAFF haplotypes. While the CTAT haplotype was associated with Ro/La-positive pSS [odds ratio (OR) 2.6; 95% CI 1.7, 4.1; $P = 0.00004$], the TTTT haplotype was associated with elevated s-BAFF in autoantibody-positive pSS ($n = 85$; 88% females; $P = 0.008$). The shared -871 T allele had no independent contribution to disease susceptibility or s-BAFF.
Conclusions. Disease susceptibility for Ro/La-positive pSS is increased with the CTAT haplotype, but not associated with high s-BAFF levels. Elevated s-BAFF levels in pSS are associated with the TTTT haplotype and may be a secondary phenomenon in Ro/La-positive pSS. While both haplotypes carry the -871 T allele, this allele is not independently associated with disease susceptibility.

Key words: B-lymphocyte activating factor, Sjögren's syndrome, Polymorphism.

Introduction

The importance of B cells in primary SS (pSS) is underscored by the presence of hypergammaglobulinaemia and autoantibodies against Ro and La antigens, an increased risk for development of B-cell lymphomas, and more recently by the efficacy of B-cell depleting therapy by monoclonal antibodies against the mature B-cell membrane antigen CD20 [1–5]. However, the exact manner in which B cells contribute to pSS remains unclear. B-lymphocyte activating factor (BAFF, TNFSF13B), a member of the TNF superfamily, is an essential cytokine for B-cell survival. BAFF deficiency leads to B-cell depletion, while transgenic mice over-expressing BAFF develop systemic autoimmune disease reminiscent of both SLE and pSS [6–9]. In humans, serum BAFF (s-BAFF) levels are increased in subsets of pSS patients and correlate with autoantibody levels [10, 11], while BAFF expression is up-regulated in labial tissue of pSS patients [12]. Therefore, dysregulation of BAFF expression may be a critical element in the chain of events leading to autoimmunity.

Genetic background is an important factor in the development and expression of systemic autoimmune disease [13]. Single nucleotide polymorphisms (SNPs) in the 5′ regulatory region of the *BAFF* gene (13q32–34) have been identified in patients with SLE and RA [14] and genetic defects in BAFF receptors (TACI and BAFF-R) have been described in common variable immunodeficiency [14, 15]. Subsequent interest has centered on the *BAFF* -871 C→T SNP that was associated with anti-Sm autoantibodies in Japanese SLE patients [14]. In a more recent study, only the -871 T→C SNP was detected by sequencing the entire *BAFF* gene in a French pSS cohort [16]. This SNP was not associated with pSS disease susceptibility, although the T allele was associated with higher s-BAFF levels in pSS patients. These findings were surprising given there are other relatively high-frequency SNPs in this region. We therefore performed a case–control study to investigate additional SNPs in the extended 5′ regulatory region of the *BAFF* gene, and their association with disease susceptibility and expression in an Australian Caucasian pSS cohort.

Materials and methods

Study participants

One hundred and thirty-six Caucasian population-based controls (53% females, median age 56 yrs) and 123 Caucasian pSS patients (90% females, median age 58 yrs) from the South Australian SS research registry were included in the study. All patients met the revised 2002 American–European consensus research classification criteria for pSS [17]. The study was conducted in accordance with the Declaration of Helsinki and approved by the Human Ethics Committee of The Queen Elizabeth and Royal Adelaide Hospitals, and all patients gave informed, written consent.

Serology

Serum levels of immunoglobulin G (IgG) (calorimetry), IgM RF (nephelometry) and Ro/La autoantibodies were measured as part of a standard diagnostic procedure, although IgG and RF levels were not available for all patients. Anti-Ro/La autoantibody specificity was determined by ELISA using recombinant Ro60, Ro52 and La proteins and sera from patients with anti-La were further tested by counterimmunoelectrophoresis (CIEP) as previously described [18]. Of the 123 pSS patients, 28 (23%) were negative and 95 (77%) positive for anti-Ro/La autoantibodies. Seropositive Ro + La patients by ELISA were further subdivided into non-precipitating La, i.e. Ro + La (ppt−), or precipitating, i.e. Ro + La (ppt +), on the basis of a precipitin line formed by anti-La antibodies on CIEP. Therefore, in addition to the seronegative subset, seropositive pSS patients were classified into one of the three serological subsets: anti-Ro alone (15/123 = 12%),

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anti-Ro+La (ppt−) (21/123 = 17%) and anti-Ro + La (ppt+) (59/123 = 48%). These subgroups are characterized by increasing titre, a more polyclonal autoantibody response, higher RF and IgG levels, and differing MHC associations [18, 19].

Stored serum for BAFF measurement was available for 112 pSS patients (90% female) and 59 (89% female) age-matched controls. The s-BAFF was measured by a quantitative sandwich enzyme immunoassay (Quantikine Human BAFF Immunoassay; R&D Systems, Minneapolis, MN, USA). In short, polystyrene microplate wells were coated with a mouse monoclonal antibody against BAFF, and after incubation with serum, the assay was developed with a polyclonal second antibody against BAFF conjugated to horseradish peroxidase. After a wash to remove unbound antibody–enzyme reagent, a substrate solution was added to develop colour in proportion to the amount of bound BAFF. After stopping the development of colour, intensity was then measured by an automated microplate reader at 450 nm (EMax, Molecular Devices Corporation, Sunnyvale, CA, USA) with background correction. The manufacturer's recommendations were followed throughout and standard curves were derived from serial dilutions of 40 ng of recombinant human BAFF and negative (H_2O) controls were included in each run. All measurements were done in duplicate and results were averaged; intra-assay variation was between 4% and 16%.

BAFF genotyping

SNPs within ±5 kb of the start of the *BAFF* gene (chromosome 13, 107715–107725 K) were analysed in Caucasian (CEU) family data from the HapMap project (www.hapmap.org) using Haploview software [20]. This analysis identified a single haplotype block in the 5′ regulatory region of *BAFF*, with four common haplotypes tagged by SNPs rs9514827, rs3759467, rs9514828 (Fig. 1A). Of note, SNP rs9514828 is the -871 C→T SNP previously associated with elevated s-BAFF levels [14, 16], and this SNP is carried on two of the four common *BAFF* haplotypes. A fourth SNP, rs1041569, not included in the HapMap data, but in close proximity to rs3759467, was also selected because these two SNPs are in close proximity to a putative androgen/progestin transcription factor binding site. DNA was extracted from peripheral whole blood of patients and controls, using the salt precipitation method and genotyping was performed for these four biallelic SNPs (Fig. 1B) in the 5′ regulatory region of the BAFF gene: -2841 T→C (rs9514827), -2704 T→C (rs3759467), -2701 T→A (rs1041569), -871 C→T (rs9514828) by PCR-RFLP (See Supplementary Table 1, available as supplementary data at *Rheumatology* Online). All PCR reactions were performed in 20 μl volumes, with an annealing temperature of 55°C. PCR products were digested with 2 U of the appropriate restriction enzyme for 4 h at the manufacturer's recommended temperature, and restriction fragments were electrophoresed on 2% agarose gels with sodium borate buffer [21], stained with ethidium bromide and visualized under UV light. Direct sequencing was performed for 20 samples to validate the genotyping by PCR-RFLP and reference samples of each genotype were included in each assay.

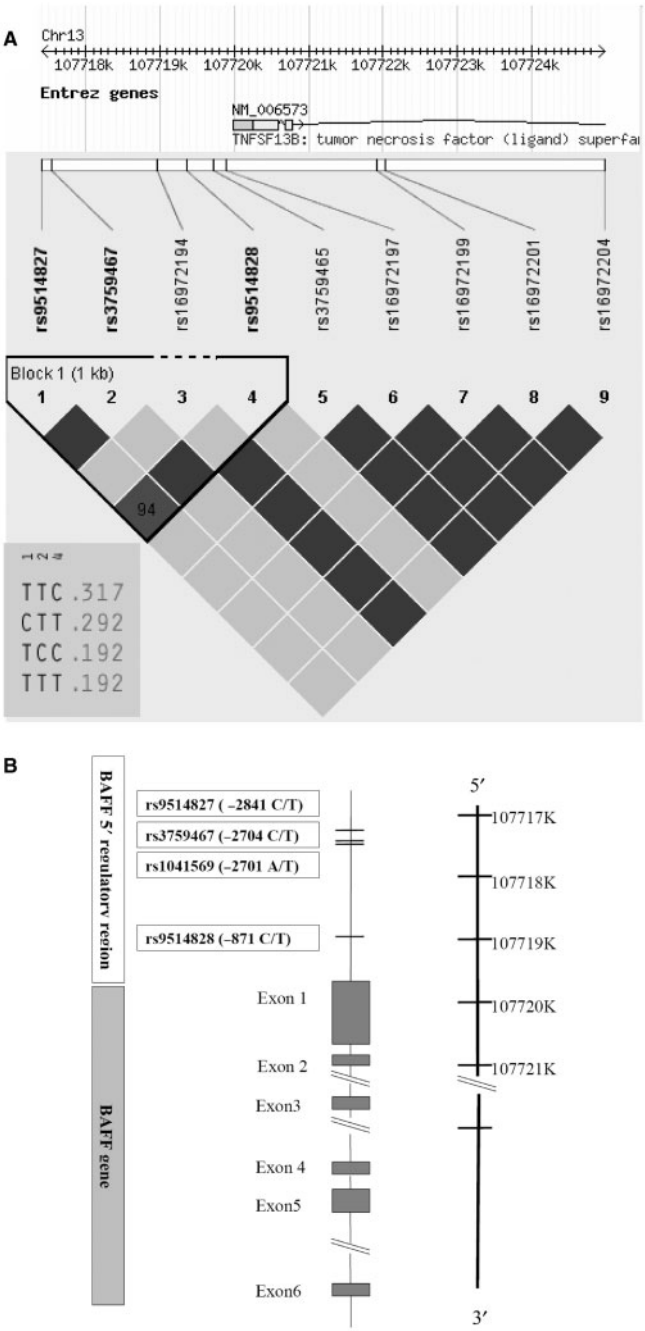

FIG. 1. (**A**) Definition of the haplotype block in the 5′ regulatory region of the *BAFF* gene on chromosome 13 q32–34 identified from Caucasian (CEU) families in the HapMap (www.hapmap.org) project. (**B**) Location of the four SNPS genotyped in this study on chromosome 13 in relation to the coding region. Three SNPs were identified as tagging SNPs from HapMap, and one additional SNP (-2701 T→A, rs1041569) because of proximity to a putative androgen/progestin transcription factor binding site.

Statistics

Correlations between s-BAFF, IgG and RF were performed using the Spearman rank correlation coefficient; otherwise, analyses were performed using a generalized linear model (GLM) regression framework. Global *P*-values were derived from the log-likelihood ratio chi-squared test, specific *P*-values from the significance of the regression coefficient and *P*-values <0.05 were considered indicative of statistical significance.

s-BAFF, IgG, IgM-RF were not normally distributed and were therefore transformed prior to analysis. Log transformations of BAFF and IgG levels essentially normalized the distributions, while the RF distribution was normalized by cubed root transformation (i.e. $RF^{(1/3)}$). The reported 'means' and 95% CIs are therefore back transformations of the regression coefficients. Comparison of s-BAFF levels between pSS patients and controls were analysed by analysis of variance-style normal regression, whereas proportional odds regression was used for analysis of an ordinal trend in s-BAFF, IgG and RF within pSS Ro/La autoantibody subgroups. These analyses were performed using Statistica v6.1 (Statsoft Inc., Tulsa, OK, USA).

BAFF haplotypes, defined by the four SNPs, were analysed using the freely available hapassoc v1.1 software (Simon Fraser University, Burnaby, BC, Canada) [22], assuming an additive

genetic model, and rare haplotypes (frequency <5%) were pooled for analysis purposes. Briefly, haplotypes were estimated by the Expectation–Maximization (EM) algorithm, and continuous or categorical trait associations were then analysed in a GLM regression framework, incorporating any uncertainty associated with an individual's exact haplotype assignment. The association between BAFF haplotypes and pSS was analysed by logistic regression, and odds ratios (ORs) for individual haplotypes derived from the regression coefficients. Associations between BAFF haplotypes and s-BAFF, IgG, RF (transformed data) were analysed by normal regression. To enhance interpretation for these analyses, the fitted mean levels (95% CI) for each homozygous genotype were estimated from the regression coefficients.

Results

s-BAFF is elevated in Ro/La autoantibody pSS

Mean s-BAFF concentrations were 1770 pg/ml (95% CI 1570, 1996; $n=85$) in Ro/La-positive pSS patients, 1193 pg/ml (95% CI 964, 1478; $n=27$), in Ro/La-negative pSS and 1171 pg/ml (95% CI 1016, 1350; $n=59$) in age- and gender-matched controls. Therefore, s-BAFF levels were specifically elevated in Ro/La-positive pSS patients ($P=0.0002$), but levels in autoantibody-negative pSS patients were equivalent to that in controls. Further, s-BAFF levels were highest in Ro + La (ppt+) pSS patients (Fig. 2A). As previously reported [19], RF and IgG levels show a similar pattern (Fig. 2B and C). The s-BAFF, RF and IgG are only substantially elevated in Ro/La-positive pSS; however, no significant correlation was observed between s-BAFF levels and IgG levels (r −0.14, $P=0.17$), or RF levels (r 0.13, $P=0.26$), in these patients.

There was no evidence of an effect of age on s-BAFF levels in either pSS patients or controls (data not shown), and there were insufficient data to evaluate the effect of gender, because the majority of samples were from females.

BAFF CTAT haplotype is associated with susceptibility to Ro/La-positive pSS

The four BAFF SNPs were in strong linkage disequilibrium ($P<0.000001$) and formed four common (frequency >5%) haplotypes. These haplotypes were identical, and comparable in frequency, to those identified in the HapMap analysis, and the additional SNP included in this study, rs1041569, did not further discriminate between haplotypes.

The *BAFF* haplotype frequency distributions were significantly different between pSS patients and controls (Table 1, global P-value = 0.015). This difference was attributable to an increase in frequency of the CTAT haplotype (haplotype 2) in pSS patients with an associated OR of 2.12 (95% CI 1.27, 3.55; $P=0.004$) relative to the TTAC haplotype, the most frequent haplotype in the controls. Because pSS patients are predominantly female, a gender-adjusted analysis was also performed. As expected, the results of this analysis were essentially identical (OR 2.40; 95% CI 1.35, 4.26; $P=0.003$).

The pSS-associated CTAT haplotype carries the -871 T allele; however, there was no evidence of any association with the TTTT haplotype (haplotype 4, $P=0.65$), which also carries this allele. In fact, when considered on its own, there was no association between the -871 C→T SNP and pSS ($P=0.57$), which is consistent with previous studies [14, 16]. Further, the CTAT haplotype association was apparently specific for Ro/La autoantibody-positive pSS patients (OR = 2.59; 95% CI 1.65, 4.07; $P=0.00004$, Table 2) and the frequency of this haplotype was even somewhat decreased in Ro/La-negative pSS patients (OR = 0.47; 95% CI 0.17, 1.35; $P=0.16$).

There were no differences in haplotype frequencies between the three pSS autoantibody-positive subgroups ($P=0.83$), although low numbers limited the power of this analysis. In addition, there was no evidence of any epistatic interactions between *BAFF* haplotypes and HLA *DRB1* alleles, which are also associated with Ro/La autoantibody-positive pSS [19].

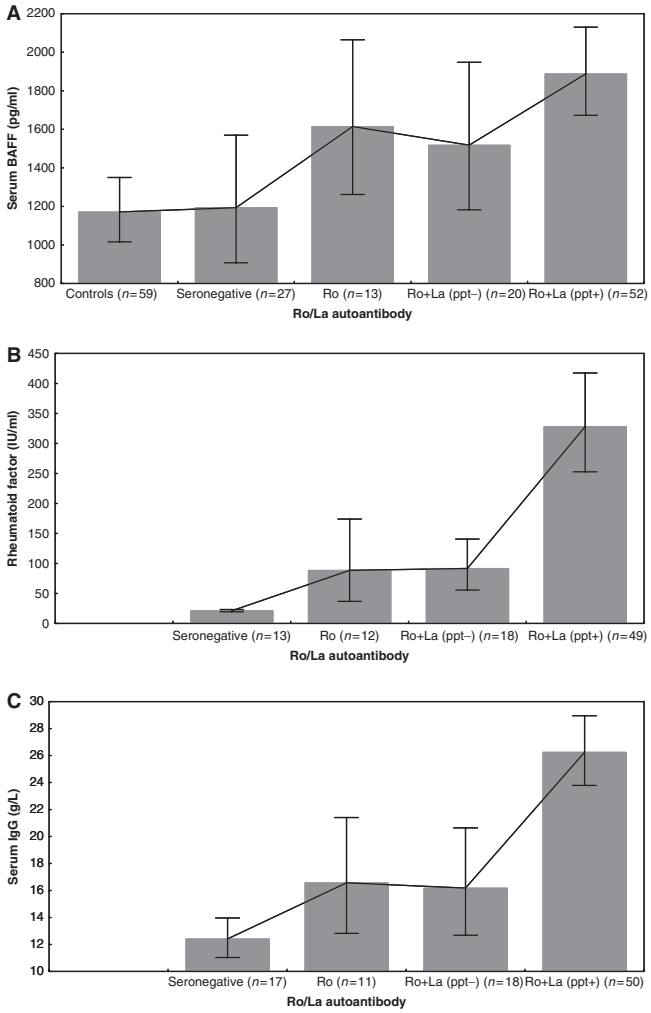

FIG. 2. Relation between median serum levels of (**A**) BAFF, (**B**) RF and (**C**) IgG and increasing diversification of the autoantibody response in patients with pSS. Seropositive Ro + La patients by ELISA were further subdivided into non-precipitating La, i.e. Ro + La (ppt−), or precipitating i.e. Ro + La (ppt+), on the basis of a precipitin line formed by anti-La antibodies on CIEP. These subgroups are characterized by increasing titre, a more polyclonal autoantibody response, and differing MHC associations [18, 19]. *P*-values for an ordinal trend were derived by ordinal proportional OR regression. Variation in numbers in subgroups reflects serum availability (see Materials and methods section). Vertical bars represent 95% CI.

TABLE 1. BAFF haplotype frequencies in pSS patients and controls

	Haplotype[a]	pSS ($n=123$)	Controls ($n=136$)	OR (95% CI)	*P*
(1)	hTTAC	0.257	0.302	1	
(2)	hCTAT	0.336	0.213	2.12 (1.27, 3.55)	0.004
(3)	hTCAC	0.140	0.134	1.39 (0.74, 2.64)	0.31
(4)	hTTTT	0.125	0.174	0.87 (0.49, 1.56)	0.65
	Rare_pool	0.143	0.176	1.03 (0.58, 1.84)	0.91
		Global test: $\chi 2=12.39$. df = 4, $P=0.015$			

[a]Haplotype order: rs9514827_rs3759467_rs1041569_rs9514828. Note rs1041569 was not included in the original HapMap analysis (Fig. 1A).

BAFF TTTT haplotype influences s-BAFF levels in both Ro/La-positive pSS patients and controls

Previous studies have identified that the *BAFF* -871 T allele is associated with elevated s-BAFF levels in autoimmune

TABLE 2. The *BAFF* CTAT haplotype frequency in Ro/La-negative and -positive pSS patients compared with controls

CTAT haplotype	Ro/La-negative pSS ($n=28$)	Ro/La-positive pSS ($n=95$)	Controls ($n=136$)
Frequency	0.143	0.393	0.213
OR (95% CI)	0.47 (0.17, 1.35)	2.59 (1.65, 4.07)	1
P-value	0.16	0.00004	–

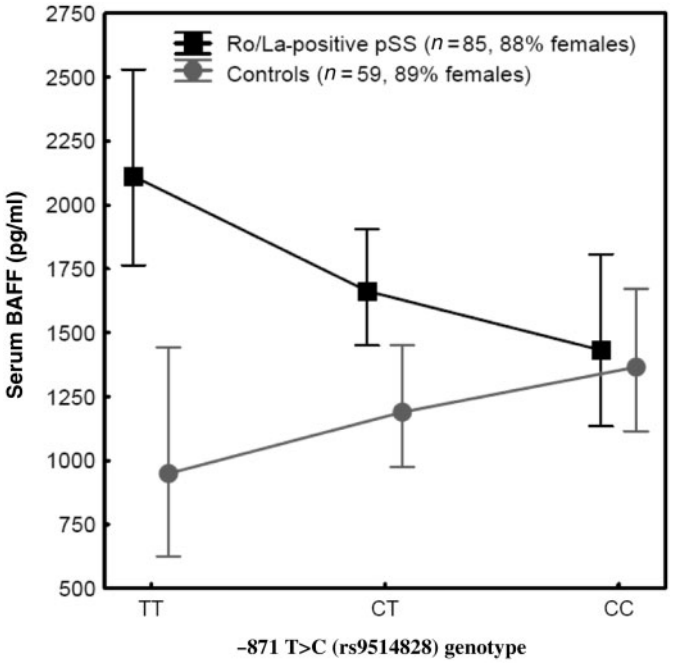

FIG. 3. Median s-BAFF by -871 C→T SNP genotype in both pSS patients and controls. Vertical bars represent 95% CI.

patients [14, 16]. This effect was also observed in Ro/La autoantibody-positive pSS patients in this study, where the T allele was associated with higher s-BAFF levels in a dose-dependent manner (Fig. 3). However, a converse effect in age- and gender-matched controls, not previously studied, was also observed, whereby the -871 T allele was associated with lower BAFF levels in controls. This is suggestive of genetically controlled differences in BAFF regulation in health and autoimmune disease.

The *BAFF* -871 T allele is carried on two of the four common *BAFF* haplotypes, CTAT and TTTT. We therefore investigated and compared the effects of the -871 T allele and these two haplotypes on s-BAFF levels by additive genetic regression analysis (Table 3). Because the s-BAFF levels were $\log_e$ transformed prior to the analysis, exponentiation of the regression coefficients has interpretation as the proportional change. In controls, the -871 T allele was associated with 17% lower (95% CI 2, 29; $P=0.029$) s-BAFF. This was mirrored by the TTTT haplotype, associated with 20% lower (95% CI 3, 34; $P=0.022$) s-BAFF, and there was no evidence of an effect of the CTAT haplotype ($P=0.98$). In Ro/La autoantibody-positive pSS patients, the -871 T allele was associated with 22% (95% CI 4, 44; $P=0.013$) higher s-BAFF levels, also mirrored by the TTTT haplotype, associated with 35% higher (95% CI 8, 68; $P=0.008$) s-BAFF. Again there was no effect of the CTAT haplotype ($P=0.13$). Therefore, the opposite effects of the -871 T allele on s-BAFF levels in both pSS patients and controls can be attributed specifically to the TTTT haplotype, and these opposite effects substantially account for the differences in s-BAFF levels between pSS patients and controls. There was no evidence that the -871 T allele, when carried on the pSS-associated CTAT haplotype, had any influence on s-BAFF levels. Further,

TABLE 3. Comparison of the effects of the -871 (rs9514828) T allele and haplotypes that carry this allele on $\log_e$ serum BAFF levels in Ro/La autoantibody-positive pSS patients and controls

	Coefficient	S.E	*P*
-871 T allele			
Intercept	7.237	0.107	0.000
-871 T allele: controls	−0.182	0.083	0.029
pSS	−0.002	0.153	0.99
-871 T allele: pSS	0.384	0.116	0.001
-871 T haplotypes			
Intercept	7.192	0.082	0.000
hCTA<u>T</u>: controls	−0.002	0.092	0.98
hTTT<u>T</u>: controls	−0.224	0.098	0.022
pSS	0.119	0.118	0.31
hCTA<u>T</u>: pSS	0.110	0.116	0.34
hTTT<u>T</u>: pSS	0.522	0.149	0.0005

Analysis was performed by regression analysis assuming a genetic additive model. The effect of the -871 T allele on $\log_e$ serum BAFF levels in Ro/La autoantibody-positive pSS patients is specifically attributed to the TTT<u>T</u> haplotype. There is no effect of the CTA<u>T</u> haplotype, which also carries this allele.

there was no evidence that other haplotypes were associated with s-BAFF in either pSS patients or controls (data not shown).

Discussion

In this study, we have confirmed elevated s-BAFF levels in pSS, with levels comparable with that seen in other studies using the same assay [23, 24]. s-BAFF levels were elevated specifically in Ro/La autoantibody-positive pSS patients (mean 1770 pg/ml), whereas s-BAFF in autoantibody-negative pSS patients was comparable with that in age- and gender-matched controls (1193 *vs* 1171 pg/ml, respectively). Further, s-BAFF levels were highest in Ro + La (ppt+) pSS patients, in which there is the greatest diversification of the Ro/La autoantibody response. Our results are therefore consistent with previous studies reporting correlations between s-BAFF and autoantibody titre in pSS [11], SLE and RA [24]. Similar to s-BAFF, RF and IgG levels were only elevated in Ro/La autoantibody-positive pSS patients, and also were highest in Ro + La (ppt+) pSS patients. It is possible that these similar trends may result from RF interference on s-BAFF ELISA quantitation. However, this is unlikely, as there was no correlation between either RF or IgG with s-BAFF in individual Ro/La autoantibody-positive patients. Previous studies have reported mixed findings on a correlation between s-BAFF and RF [11, 16, 24]. In our data, an artificial correlation could be induced by including autoantibody negative patients (who have both normal, instead of elevated, s-BAFF and RF) into the analysis, suggesting that the inclusion (and proportion) of autoantibody-negative patients may be an important confounder in this question.

Genetic polymorphisms may be one of the driving forces behind the increased BAFF expression that is seen in subsets of patients with systemic autoimmune diseases [25, 26]. The coding region of the *BAFF* gene may be relatively conserved [14, 16], but polymorphisms in the *BAFF* 5′ regulatory region have previously been reported in patients with SLE and RA [14]. While none of the three *BAFF* haplotypes described in that study were related to disease susceptibility in Japanese patients, the T allele of the -871 C→T SNP was associated with BAFF m-RNA levels in monocytes and the SLE-specific anti-Sm antibody response. In a more recent study, only the -871 C→T SNP was detected in a French pSS cohort, and although not associated with pSS disease susceptibility, the T allele was associated with increased s-BAFF levels in pSS patients [16].

The present study investigated three novel SNPs in the 5′ regulatory region of the *BAFF* gene, encompassing 2.8 kb upstream from exon 1 (Fig. 1), in addition to the -871 C→T SNP, which formed a single haplotype block, identified by HapMap analysis, with four common haplotypes. We identified two *BAFF*

haplotypes, which were of interest in Ro/La autoantibody-positive Caucasian pSS patients. The CTAT haplotype was associated with disease susceptibility. While this haplotype was increased in frequency in the pSS cohort as a whole, it was clear that this association was primarily with Ro/La autoantibody-positive patients (OR = 2.59; 95% CI 1.65, 4.07; $P = 0.00004$) and the frequency of this haplotype was even somewhat decreased in Ro/La-negative pSS patients (OR = 0.47; 95% CI 0.17, 1.35; $P = 0.16$). In contrast, the TTTT haplotype was associated with increased levels of s-BAFF and RF in Ro/La-positive pSS patients, but surprisingly, lower s-BAFF levels in controls. This haplotype may substantively account for the differences in s-BAFF levels between pSS patients and controls, and is suggestive of genetically related up-regulation of BAFF in Ro/La autoantibody-positive pSS.

Both the CTA<u>T</u> and TTT<u>T</u> haplotype carry the -871 T allele, which has been associated with increased s-BAFF levels in the previous studies, but was unrelated to disease susceptibility for SLE or pSS [14, 16]. Our results are entirely consistent with these studies. Therefore, while there was no overall association with the -871 T allele and pSS, as previously reported, we did observe a specific association with the CTAT haplotype. As expected, we observed an association between s-BAFF levels and the -871 T allele; however, this was attributable specifically to the TTTT haplotype and there were no substantial effects of the CTAT haplotype. Therefore, it is unlikely that the -871 T allele is a sole, major determinant of s-BAFF levels. Genetic variation within populations is inherently structured into haplotypes [27, 28] which represent combined, functional polymorphic units that are inherited *en bloc*. Haplotypes may also tag other regional polymorphic sites, and are therefore, as the current study demonstrates, considered more robust than isolated SNPs in unravelling the genetic background of complex disease [27, 28].

If genetically determined elevated s-BAFF were a primary risk factor for development of autoantibody-positive pSS, then a common genetic background would be expected for both disease susceptibility and elevated s-BAFF levels. However, the diverging effects of the CTAT and TTTT haplotypes on anti Ro/La-positive disease susceptibility and serum BAFF levels are not compatible with this. It is therefore possible that elevated s-BAFF levels observed in pSS may be a secondary phenomenon associated with autoantibody-mediated disease. A potential mechanism for elevated s-BAFF levels in pSS is activation of the type I interferon system, which occurs in autoantibody-positive pSS [29]. Previous *in vitro* studies have indeed demonstrated that BAFF is expressed in response to IFN-α stimulation in pSS [30, 31].

The clinical significance of elevated s-BAFF in pSS is unknown. BAFF is thought to promote autoimmunity through reducing the apoptotic propensity of autoreactive B cells. However, BAFF has pleiotropic effects on mature human B cells, and can also play an inhibitory role in B-cell differentiation by providing regulatory signals during specific T cell-independent events, which protect the balance between memory B cells and Ig-secreting cells outside germinal centres [32]. Further, s-BAFF levels increase in response to anti-TNF or anti-CD20 therapy [33]. Therefore, the role of BAFF in autoimmune diseases is complex.

The mechanism for the association between the *BAFF* CTAT haplotype and Ro/La autoantibody-positive pSS is not clear. While there was a lack of influence on s-BAFF levels, this does not preclude an effect of this haplotype on membrane BAFF expression and this will be investigated in future studies. There are several explanations possible for the up-regulation of s-BAFF levels in pSS patients in association with the TTTT haplotype. First, the novel SNPs in this study lie within a putative androgen/progestin transcription factor binding site, which may be specific for the T<u>T</u>TT haplotype. It is possible that the s-BAFF up-regulation in pSS may be gender specific, since the majority (~90%) of patients and controls for whom s-BAFF levels were available were females, and sex hormone imbalances have been reported in pSS [34]. Second, the transcription factors Nuclear factor-kappa B (NF-κB) and Nuclear factor of activated T cells (NFAT) are involved in regulating BAFF expression through specific binding sites in the BAFF 5′ regulatory region [35]. NF-κB is constitutively activated in SLE and RA [36] and could possibly be activated in pSS as well. The theoretical binding sites for NF-κB and NFAT lie very close to the -871T SNP [35] and haplotypic differences could thus influence transcription factor binding. Finally, IFN-α induces BAFF expression in salivary gland epithelial cells from pSS patients [31]. Given the central role that is now proposed for IFN-α in autoantibody-positive pSS disease pathogenesis [31], we speculate that the results of this study may be largely explained by haplotypic controlled differences in the BAFF response to IFN stimulation.

Patients with pSS have an increased risk of B-cell non-Hodgkin lymphoma (NHL) [37, 38]. BAFF neutralization attenuates NHL B-cell survival [39], and BAFF levels are correlated with NHL disease severity and outcomes [40]. While beyond the scope of this study, elevated BAFF may be an important susceptibility factor for NHL risk in pSS patients, and future studies will be of interest.

In summary, we have identified haplotypes in the 5′ regulatory region of the *BAFF* gene, which are associated with both disease susceptibility and s-BAFF levels in anti-Ro/La-positive pSS patients. Further studies are required to extend these findings to other pSS patient cohorts, other autoimmune diseases such as SLE and RA, and to the risk of NHL.

Rheumatology key messages

- Two haplotypes in the 5′ regulatory region of the *BAFF* gene have independent associations with susceptibility to autoantibody-positive pSS and elevated s-BAFF levels.
- The contribution of *BAFF* polymorphisms to pSS susceptibility is not simply attributable to s-BAFF levels.

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Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

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