Role of APRIL (TNFSF13) polymorphisms in the susceptibility to systemic lupus erythematosus in Japanese

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Objectives. A polymorphism of APRIL, c.199G>A (Gly67Arg), has been reported to be associated with systemic lupus erythematosus (SLE) in Japanese. To identify the causative polymorphism, we screened for polymorphisms of APRIL as well as TWEAK (TNFSF12), a closely located gene that generates a fusion protein TWE-PRIL by intergenic splicing. Association of APRIL and TWEAK with rheumatoid arthritis (RA) was examined in parallel.

Methods. Polymorphisms were screened by direct sequencing. Association was analysed by case-control analysis using 266 SLE, 298 RA and 208 healthy individuals. Allele-specific difference in the mRNA level was examined using RNA difference plot analysis. Serum APRIL level, the frequency of plasma cells in the spleen, and inhibited the IgM response to a T cell-dependent antigen in NZB/W F1 mice [16]. Blys/APRIL pathway is strongly implicated in autoimmunity. Blys-transgenic and Taci-deficient mice developed symptoms resembling systemic lupus erythematosus (SLE) [5, 17–19]. Treatment with soluble BAF-F-R and TACI-inhibited murine SLE and collagen-induced arthritis [5, 16, 20–22]. Furthermore, serum and synovial fluid BLYS and APRIL levels in patients with SLE or rheumatoid arthritis (RA) have been reported to be increased [23–26]. Genome-wide studies revealed linkages of the chromosomal regions 13q32, 17p12-q11 and 17p13 with SLE or rheumatoid arthritis (RA) have been reported to be increased [23–26]. Genome-wide studies revealed linkages of the chromosomal regions 13q32, 17p12-q11 and 17p13 with SLE [27–30]. These regions contain the genes encoding BLYS (13q32), APRIL (17p13.1) and TACI (17p11.2).

These findings led us to consider BLYS, APRIL and their receptors as strong candidate susceptibility genes to autoimmune diseases. Thus far we conducted polymorphism screening and association studies of BCMA and BLYS [31, 32], and reported that a promoter polymorphism of BLYS was associated with increased mRNA level and slightly overrepresented in anti-Sm positive SLE. As for APRIL, an association of a single-nucleotide polymorphism (SNP) c.199G>A (Gly67Arg, rs11552708) with SLE was reported in Japanese; namely, c.199A/G homozygotes were significantly decreased in SLE [33]. APRIL is processed intracellularly by furin convertase and secreted as a soluble form, and because position 67 is more proximally located than the cleavage site, it cannot be involved in the binding with its receptors. Therefore, the mechanism of association remains unknown.

The gene coding for TWEAK (TNFSF12) is located on chromosome 17p13 within less than 1 kb telomeric to APRIL. TWEAK stimulates production of pro-inflammatory molecules in fibroblasts and synoviocytes [34], and induces angiogenesis [35]. TWEAK expression was reported to be upregulated in SLE T cells [36]. A fusion protein between TWEAK and APRIL generated by intergenic splicing has been identified and termed TWE-PRIL...
TWE-PRIL is composed of cytoplasmic and transmembrane domains of TWEAK fused to the APRIL receptor-binding domain, expressed on T cells and monocytic cell lines in a membrane-bound form, and has been shown to be biologically active. These lines of evidence indicate that TWEAK polymorphisms should also be examined for association with SLE to address the question whether APRIL SNP is functionally responsible for the association or merely reflects linkage disequilibrium (LD) with TWEAK. Information on this region available from the HapMap database is very limited.

Patients were recruited at Juntendo University, Matsuta Clinic and University of Tokyo Hospital, all in Tokyo. Diagnoses and classification into subsets were made according to the American College of Rheumatology criteria for SLE and RA [38, 39]. The healthy controls were recruited at the University of Tokyo, Japanese Red Cross Central Blood Center and Kanagawa Red Cross Blood Center. All patients and healthy controls were unrelated Japanese, living in the Tokyo area. Informed consent was obtained from all donors. This study design was reviewed and approved by the research ethics committees of the University of Tokyo and Juntendo University.

Polymorphism screening and genotyping

Polymorphism screening was performed by direct sequencing of genomic DNA of 32 (for APRIL) and 16 (for TWEAK) subjects. Genotyping for the association studies was conducted using PCR—single strand conformation polymorphism (SSCP) and direct sequencing. PCR was performed using AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) by a T Gradient thermocycler (Biometra, Göttingen, Germany). PCR primers and SSCP conditions are listed in Supplementary Table 1. Direct sequencing was carried out using a BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

RNA difference plot (RDP) analysis

Allele-specific mRNA levels of APRIL were compared between c.*263C and T (n = 4), c.199G and A (n = 7), and c.287A and G (n = 4), using CDNA and genomic DNA extracted from peripheral blood mononuclear cells (PBMCs) of heterozygous healthy individuals.

The fragments encompassing the polymorphic sites were amplified using cDNA and genomic DNA as templates. After PCR, both alleles were distinguished by SSCP using SF5200 auto sequencer (Hitachi Ltd, Tokyo, Japan) as previously described [40]. Electrophoresis was carried out at 20°C, 30 W for 2 h. The signal intensity of the polymorphic fragments was analysed using the software program Allele Links (Hitachi Ltd).

‘RNA ratio’ was calculated by dividing the signal intensity of one allele by that of the other allele by using cDNA as the template. ‘DNA ratio’ was similarly obtained by using genomic DNA as the template. To correct for the amplification bias, expression ratio (ER) was calculated by dividing the RNA ratio by the DNA ratio.

Enzyme-linked immunosorbent assay (ELISA)

Serum APRIL protein levels were determined in 22 healthy individuals using human APRIL ELISA (Bender MedSystems, Vienna, Austria) following the manufacturer’s instructions. The detection limit of serum APRIL was 0.8 ng/ml. Samples were analysed in duplicate.

This ELISA system did not cross-react with recombinant human BAFF (Oncogene Research Products, Cambridge, MA, USA) at the concentration between 0.78 and 100 ng/ml.

Statistical analysis

Association was evaluated by \( \chi^2 \) test for 2 \times 2 and 2 \times 3 contingency tables. When one or more of the variables in the contingency tables was 5 or less, Fisher’s exact test was employed.

### Methods

#### Subjects

Genomic DNA extracted from peripheral blood leucocytes using QIAamp blood kit (QIAGEN, Hilden, Germany) was used for the analysis of APRIL. For TWEAK, whole genome amplification products were used. Whole genome amplification was carried out using the GenomiPhi DNA Amplification Kit (Amersham Biosciences, Piscataway, NJ, USA), following the manufacturer’s instructions.

### DNA samples

### Table 1. Minor allele frequencies of APRIL and TWEAK polymorphisms in patients with SLE, RA and healthy controls

| Polymorphisms | rs number | SLE \( n = 532 \) | RA \( n = 596 \) | Control \( n = 416 \) |
|---------------|-----------|-----------------|----------------|----------------|---|
| APRIL         |           |                 |                |                |---|
| -888C > T    | rs424613  | 58              | (10.9)         | 65             | (10.9) | 50 (12.0) |
| c.199G > A    | rs1152708 | 187             | (35.0)         | 236            | (39.6) | 176 (42.3) |
| c.585C > C    | rs6008    | 63              | (12.4)         | 63             | (10.6) | 33 (7.9) |
| TWEAK         |           |                 |                |                |---|
| c.600G > C    | rs308300  | 341             | (62.0)         | 410            | (68.8) | 294 (70.7) |
| c.504 > A     | rs5417    | 108             | (20.3)         | 121            | (20.3) | 64 (15.4) |
| c.293C > T    | rs6608    | 123             | (22.2)         | 63             | (10.6) | 33 (7.9) |

### Notes

- Frequencies of major allele (c.287G) are shown.
- \( P = 0.004, P^* = 0.006, P^* = 0.025, P^* = 0.046 \) values were calculated by \( \chi^2 \) test from 2 \times 2 contingency table.
- Numbers in the parentheses indicate the percentage. The nucleotide sequences of the new variations were registered in the DDBJ nucleotide sequence database with the accession numbers AB222992 and AB222993. The nomenclature was based on den Dunnen et al. [42].
In addition, the association between three SNPs (c.199G > A, c.287A > G and c.*263C > T) and SLE was examined under three different genetic models (recessive, dominant and co-dominant) by logistic regression analysis. Assuming a polymorphic site with two alleles A and a, to test a recessive model for the A allele in the logistic regression analysis, genotypes were encoded as 0 = aa or Aa and 1 = AA. To study a dominant model for the A allele, genotypes were encoded as 0 = aa and 1 = AA or Aa. To study a co-dominant model for the A allele, genotypes were encoded as 0 = aa, 1 = Aa, and 2 = AA. In multivariate logistic regression analysis, the same genetic model was applied to all the SNPs to be evaluated.

Haplotype frequencies and LD parameters (D' and r²) were estimated using the expectation-maximization method with PLINK software. Hardy–Weinberg equilibrium was evaluated.

For the meta-analysis of APRIL c.199G > A and c.287A > G, our data and data by Koyama et al. [33] were pooled using DerSimonian-Laird method under the random effects model [41].

Correction for multiple testing was done by Bonferroni correction. The corrected P-values (Pcorr) were obtained by multiplying the nominal P-values by the total number of association analyses performed in this study (n = 65).

Results

Association of APRIL polymorphisms with SLE

Re-sequencing of promoter, all exons and introns of APRIL in 32 individuals (10 SLE, 10 RA patients and 12 healthy controls) identified six polymorphisms, c.199G > A (Gly67Arg) and c.287A > G (Asn96Ser) in the coding region, c.*263C > T in the 3' untranslated region (UTR) and c.258+49dupG and c.504+163_504+165delACA in introns. Four variations, c.1090G > T, c.372C > T (Asn124Asn), c.603C > T (Pro201Pro) and c.643 + 85A > T, were also detected in only 1 of 64 chromosomes; however, they were excluded from analysis because of low allele frequencies. Allele frequencies at the six polymorphic sites are shown in Table 1. None of the genotypes in the case and control groups was significantly deviated from Hardy–Weinberg equilibrium.

Association analysis demonstrated that three SNPs were associated with SLE. Frequency of c.199A/A (67Arg/Arg) genotype was decreased in SLE compared with controls (10.9% vs 19.7%, odds ratio (OR) 0.50, 95% confidence interval (CI) 0.30–0.83, P = 0.0073), replicating the previous observation by Koyama et al. [33]. In addition, frequency of c.287A/A (96Asn/Asn) was increased in SLE (14.3% vs 7.7%, OR 2.00, 95% CI 1.08–3.70, P = 0.025). When our data was combined with the previous study by meta-analysis, statistical significance became much stronger for both c.199A/A (OR 0.44, 95% CI 0.29–0.67, Pcorr = 0.0001) and c.287A/A (OR 1.81, 95% CI 1.17–2.80, Pcorr = 0.008) (Table 2). Heterogeneity between two studies was not detected (P = 0.45 and P = 0.64, respectively).

These two SNPs were in LD (D' = -0.91, r² = 0.23) and three haplotypes could account for most haplotypes observed in our subjects (Table 3). The estimated haplotype frequency showed increase of c.199G-c.287A (67Gly-96Asn) and decrease of c.199A-c.287G (67Arg-96Ser) in SLE while c.199G-c.287G (67Gly-96Ser) was neutral, suggesting that both SNPs contribute to the risk of SLE (Table 3A). Such a possibility was supported by the two-locus analysis (Table 3B). The subjects with combined c.199A/A and c.287G/G (or G/A) genotype (group d) showed significantly lower risk for SLE compared with the reference group b (OR 0.54, 95% CI 0.32–0.90, P = 0.018), while those with combined c.199G/G (or G/A) and c.287A/A (group a) showed a tendency of higher risk (OR 1.80, 95% CI 0.97–3.33, P = 0.06) (Table 3B).

Furthermore, allele and allele carrier frequencies of c.*263T in the 3' UTR were increased in SLE (Tables 1 and 2). This SNP was not reported in the previous study [33], and was in not in LD with c.199G > A nor c.287A > G (Fig. 1).

We next carried out logistic regression analyses to test the association of each of the three SNPs (c.199G > A, c.287A > G and c.*263 > T) with SLE under three different genetic models (recessive, co-dominant and dominant). For c.199A, the strongest association was observed under a recessive model (P = 0.0081, OR 0.50, 95% CI 0.30–0.83). In the case of c.287A and c.*263, a co-dominant model generated the smallest P values (P = 0.0065, OR 1.44, 95% CI 1.12–1.95 and P = 0.034, OR 1.59, 95% CI 1.04–2.43, respectively). Multivariate analysis, in which all 3 SNPs were taken into account, revealed significant association of c.199A under a recessive model (P = 0.018, OR 0.53, 95% CI 0.32–0.90).

As for RA, only marginal association of intronic ACA deletion was observed (Table 1).

TWEAK polymorphisms

TWEAK polymorphisms were screened for all exons and flanking intronic regions in 16 healthy individuals, and their LD with APRIL polymorphisms was analysed in 65 healthy individuals.

Seven polymorphisms, c.72C > T (Leu24Leu) and c.600G > C (Ala200Ala) in the coding region, c.*18G > A, c.*91G > A and c.*91G > A.

Table 2. Association of APRIL c.199G > A (Gly67Arg), c.287A > G (Asn96Ser) and c.*263C > T (3' UTR) with SLE in Japanese

<table>
<thead>
<tr>
<th>SNP</th>
<th>SLE (This study)</th>
<th>SLE (Koyama et al. [33])</th>
<th>Pooled*</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.199G &gt; A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly67Arg</td>
<td>n = 266, n = 208</td>
<td>n = 148, n = 216</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>29 (10.9)</td>
<td>12 (8.1)</td>
<td>0.0036*a</td>
</tr>
<tr>
<td>G/G</td>
<td>129 (48.5)</td>
<td>76 (51.4)</td>
<td>0.44 (0.29–0.68)*</td>
</tr>
<tr>
<td>G/A</td>
<td>108 (40.6)</td>
<td>60 (40.5)</td>
<td>(0.007)</td>
</tr>
<tr>
<td>c.287A &gt; G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asn96Ser</td>
<td>n = 266, n = 208</td>
<td>n = 145, n = 200</td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>38 (14.3)</td>
<td>29 (20.0)</td>
<td>1.63 (0.87–3.03)*</td>
</tr>
<tr>
<td>G/G</td>
<td>125 (47.0)</td>
<td>50 (40.0)</td>
<td>0.008b</td>
</tr>
<tr>
<td>G/A</td>
<td>103 (38.7)</td>
<td>66 (45.5)</td>
<td>1.81 (1.17–2.80)b</td>
</tr>
<tr>
<td>c.*263C &gt; T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3' UTR</td>
<td>n = 266, n = 208</td>
<td>n = 150</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>7 (2.6)</td>
<td>2 (1.0)</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>52 (19.5)</td>
<td>29 (13.9)</td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>207 (77.9)</td>
<td>177 (85.1)</td>
<td></td>
</tr>
</tbody>
</table>

P-values, OR and 95% CI were calculated by chi² analysis for 2 x 2 contingency tables (*A/A vs (G/A and G/G), *A/A vs (G/A and G/G), *T/T and C/T vs C/C). *The two studies are combined by meta-analysis using DerSimonian-Laird method [41]. Heterogeneity was not detected between two studies. c.*263C > T was not described in the paper Koyama et al. [33].
c.*290G>T in the 3'UTR, and c.284-197T>C and c.373+233C>G in the introns were detected. In addition, two variations with low minor allele frequency, c.680G>A (Arg227His) and c.498+96T>C, were detected. LD parameters are shown in Fig. 1. Although the pair wise $D^\prime$ values were generally high among TWEAK and APRIL polymorphisms, $r^2$ values were relatively low for most combinations, owing to the difference in minor allele frequencies among the polymorphic sites.

Association of APRIL polymorphisms with clinical characteristics

We next analysed the association of APRIL c.199G>A, c.287A>G and c.*263C>T with clinical characteristics of SLE such as age of onset, presence of lupus nephritis, anti-Sm and anti-dsDNA antibodies.
All three SNPs were preferentially associated with SLE without nephritis (Supplementary Table 2). In addition, c.*263T allele was significantly increased in SLE patients positive for anti-Sm antibodies (Supplementary Table 3).

These results were confirmed by multivariate logistic regression analysis. The smallest P value for the c.199G>A association with non-renal SLE was observed under a recessive model (P = 0.0094, OR 0.35, 95% CI 0.16–0.77), while that for c.*263C>T association with anti-Sm antibody positive SLE under a co-dominant model (P = 0.0041, OR 2.77, 95% CI 1.38–5.53).

**Effects of APRIL polymorphisms on the mRNA level**

To investigate whether APRIL c.199G>A, c.287A>G and c.*263C>T were associated with mRNA levels, we employed RNA difference plot (RDP), a quantitative method to compare relative mRNA amount from two alleles in heterozygous individuals (Fig. 2) [40].

Levels of APRIL mRNA from c.*263 T allele were increased with respect to the SLE-protective c.199A/c.287G haplotype, while another haplotype was protective. The association of c.*263C>T haplotypes formed by the two non-synonymous substitutions with genotypes. Serum APRIL level was below the detection limit ELISA in 22 healthy individuals and examined their association to test this possibility, we measured APRIL serum levels by c.*263C>T intracellular transport or proteolytic cleavage. Serum level of APRIL, possibly by affecting the efficiency of amino acid substitutions might result in the difference in the mRNA levels.

**Fig. 2. Comparison of allele-specific mRNA levels of APRIL c.*263C>T by RNA difference plot (RDP) analysis.** c.*263T and C alleles were distinguished by SSCP as described previously [40]. ‘RNA ratio’ was calculated by dividing the signal intensity of T allele by that of C allele using cDNA as the template. ‘DNA ratio’ was similarly obtained by using genomic DNA as the template. To correct for the amplification bias, expression ratio (ER) was calculated by dividing the RNA ratio by the DNA ratio. mRNA and genomic DNA extracted from PBMCs of c.*263C>T heterozygous healthy individuals were used for analysis (n = 4). c.199G>A (ER = 1.05 ± 0.11, n = 7) and c.287A>G (ER = 0.94 ± 0.10, n = 4) did not reveal difference in the mRNA levels.

**Effects of APRIL polymorphisms on serum levels of APRIL**

Although the mRNA levels did not differ with respect to c.199G>A (Gly67Arg) and c.287A>G (Asn96Ser) SNPs, the amino acid substitutions might result in the difference in the serum level of APRIL, possibly by affecting the efficiency of trimer formation, intracellular transport or proteolytic cleavage. To test this possibility, we measured APRIL serum levels by ELISA in 22 healthy individuals and examined their association with genotypes. Serum APRIL level was below the detection limit ELISA in 22 healthy individuals and examined their association. c.199G>A, c.287A>G and c.*263C>T alleles were distinguished by SSCP as described previously [40].

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**Discussion**

In the present study, we confirmed the significant decrease of c.199A/A (67Arg/Arg) genotype in SLE in Japanese [33]. In addition, a number of new findings were demonstrated. We detected association of c.287A>G (Asn96Ser) and c.*263C>T (3’ UTR), only the former was in LD with c.199G>A, c.287G (ER of c.287A to G: 0.94 ± 0.10, n = 4). One of the haplotypes formed by the two non-synonymous substitutions at amino acid position 67 and 96 confers susceptibility, while another haplotype was protective. The association of c.*263C>T haplotypes formed by the two non-synonymous substitutions with genotypes. Serum APRIL level was below the detection limit ELISA in 22 healthy individuals and examined their association (Fig. 2) [40].

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**Fig. 3. Comparison of serum APRIL levels in healthy donors with respect to genotypes.** Each dot represents the mean of duplicate samples. The detection limit of this assay was 0.8 ng/ml. APRIL levels were compared among individuals with respect to the SLE-protective haplotype b (Table 3A, c.199A-c.287G (67Arg-96Ser)). All other haplotypes were combined as x. APRIL was detected in none of the six individuals with b/b diplople type, while 10 of 16 individuals with other diplople types. This difference was statistically significant (P = 0.015, Fisher’s exact test).

**Fig. 2. Comparison of allele-specific mRNA levels of APRIL c.*263C>T by RNA difference plot (RDP) analysis.** c.*263T and C alleles were distinguished by SSCP as described previously [40]. ‘RNA ratio’ was calculated by dividing the signal intensity of T allele by that of C allele using cDNA as the template. ‘DNA ratio’ was similarly obtained by using genomic DNA as the template. To correct for the amplification bias, expression ratio (ER) was calculated by dividing the RNA ratio by the DNA ratio. mRNA and genomic DNA extracted from PBMCs of c.*263C>T heterozygous healthy individuals were used for analysis (n = 4). c.199G>A (ER = 1.05 ± 0.11, n = 7) and c.287A>G (ER = 0.94 ± 0.10, n = 4) did not reveal difference in the mRNA levels.

**APRIL polymorphisms was not caused by LD with TWEAK.** Of particular interest, serum APRIL was below the detection limit in all six healthy individuals homozygous for the SLE-protective c.199A-c.287G (67Arg-96Ser) haplotype, and APRIL mRNA was elevated in the SLE-associated c.*263T allele. Serum APRIL level was reported to be increased in SLE by a Japanese group [26], although no appreciable difference was noted in a study from the USA [43]. Thus, the present study lends support to the role of APRIL, not only in the pathogenesis of, but also in the genetic predisposition to SLE, at least in Japanese.

APRIL is a type II transmembrane protein that is processed intracellularly to the Golgi apparatus by furin at 104Arg/105Ala, and the C terminal fragment is secreted as a soluble molecule [44]. Furin convertase recognizes Arg-Xaa-(Lys/Arg)-Arg as the cleavage-site sequence and also Arg-Xaa-Xaa-Arg with lower efficiency [45]. Since positions 67 and 96 are in the stalk region that remains attached to the cells after cleavage, amino acid substitutions at these positions do not affect binding with receptors. Although the crystal structure of the soluble form of mouse APRIL has been published [46], neither the structure of membrane-bound form nor that of human APRIL has been reported; therefore, information on the structure around the cleavage site is not available. However, it should be noted that position 96 is fairly close to the furin cleavage site at position 104/105. The influence of these polymorphisms on the processing of APRIL needs to be experimentally addressed in the future. In addition, since the sample size of is rather small, the association between the genotypes and serum levels need to be confirmed.

RDP analysis indicated that mRNA level of c.*263T allele was increased, while difference was not observed with respect to c.199G>A and c.287A>G polymorphisms. c.*263T was in LD with c.504+163_504+163delACA in intron 4; therefore, the possibility that the effects of this SNP may be ascribed to the intronic deletion cannot be excluded. Significant difference in serum APRIL level was not detected between four individuals with c.*263C/T and 18 with C/C genotype (data not shown). Because of the low frequency, individuals with T/T genotype could not be examined for the serum levels. Although the polymorphisms associated with mRNA level may not alter soluble APRIL level, it is possible that they may influence the surface expression level of TWE-PRIL. Although the functional
Association of APRIL polymorphisms with SLE

different characteristic between soluble APRIL and cell-associated TWE-PRIL has not been characterized, it is possible that the latter might be more relevant to the antigen-specific immune response through cognate interaction between T and B cells. Thus, these results raise a possibility that the coding and regulatory region polymorphisms, not in LD with each other, contribute to susceptibility to SLE by different mechanisms. Of note, discrepancy between blood mRNA and serum protein levels of APRIL has been reported [43], suggesting the complicated post-transcriptional and/or post-translational regulation of APRIL.

Several studies indicated a role of APRIL in autoimmunity. APRIL-transgenic mice showed expansion of B1 cells, which infiltrated in kidneys [14]. B1 cells are abundant in the peritoneal cavity, and were associated with the production of low-affinity polyreactive autoantibodies. B1 cells also have antigen-presenting activity. Expansion of B1 cells in lupus-prone mice was reported [47]. Another line of evidence indicates that APRIL-BCMA pathway is involved in plasma cell survival. BCMA, a high-affinity receptor for APRIL, has an essential role in plasma cell survival [48], and inhibition of APRIL and BLYS, but not BLYS alone, prevented survival and/or the migration of plasma cells to the bone marrow [10]. Moreover, blockade of both BLYS and APRIL, but not of BLYS alone, reduced the frequency of plasma cells in the spleens of SLE model mice [16].

Circulating CD27high plasma cells were correlated with disease activity in SLE, and higher absolute number of such cells was reported in SLE patients positive for anti-Sm, anti-dsDNA, anti-La, anti-Ro and anti-histone antibodies [49]. Plasma cells were also detected in the kidneys in SLE patients [50]. These observations suggest that APRIL may prolong the survival of plasma cells producing autoantibodies.

Analyses on the clinical characteristics revealed that c.199A/A was protective against non-renal SLE, while c.*263T was preferentially associated with anti-Sm positive SLE. Recent studies demonstrated linkages between specific chromosomal regions and clinical features of SLE. SLE multiplex families stratified by the presence of nephritis have been linked with 2q34-35 and 10q22.3 [51, 52], while the chromosomal region of APRIL (17p13) has been shown to be linked with SLE families with members of vitiligo [30]. Anti-Sm has been shown to be linked with 3q27 [53], suggesting that the genetic background associated with nephritis and anti-Sm may not be identical. Since these linkage studies are performed on Caucasian and African-American populations, the lack of linkage between anti-Sm and 17p13 may be caused by population difference, or relatively small contribution of APRIL polymorphism on the anti-Sm production. Although the mechanisms leading to the difference in the associated phenotype between c.199A/A and c.*263T require further study, it is probably related to the difference in the functional changes caused by these polymorphisms. Our data indicated that c.199A/A lead to the decrease in the soluble APRIL level, while c.*263T was associated with the elevated mRNA level. One possible scenario is that the latter leads to the increased surface expression of TWE-PRIL, which would enhance the cognate T-B interaction and facilitate anti-Sm production. In addition, it should be noted that association with clinical phenotypes is a relative one, because patients with nephritis also showed a tendency of decrease of c.199A/A, and anti-Sm-negative patients increase of c.*263T, when compared with controls, although the differences did not reach statistical significance (Supplementary Tables 2, 3).

There are some limitations in this study. The gender distribution was different between patients and controls. However, this difference is unlikely to have affected the results, because the genotypes were not different between male and female controls. In the analysis of the clinical subsets, the sample size in each group was small, and further confirmation is required.

This study provides evidence that APRIL plays a role in the genetic predisposition to SLE at least in Japanese, and implicates the biological therapeutics such as TACI-Ig or BCMA-Ig, which can block not only BLYS but also APRIL might be considered as an interesting option for a new treatment.

Rheumatology key messages

- The association of APRIL (TNFSF13) polymorphisms with susceptibility to SLE was replicated.
- Presence of susceptible and protective APRIL haplotypes was demonstrated for the first time.
- The protective haplotype is associated with decreased serum level of APRIL.

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