Role of STAT4 polymorphisms in systemic lupus erythematosus in a Japanese population: a case-control association study of the STAT1-STAT4 region

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Role of STAT4 polymorphisms in systemic lupus erythematosus in a Japanese population: a case-control association study of the STAT1-STAT4 region

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Abstract

Introduction Recent studies identified STAT4 (signal transducers and activators of transcription-4) as a susceptibility gene for systemic lupus erythematosus (SLE). STAT1 is encoded adjacent to STAT4 on 2q32.2-q32.3, upregulated in peripheral blood mononuclear cells from SLE patients, and functionally relevant to SLE. This study was conducted to test whether STAT4 is associated with SLE in a Japanese population also, to identify the risk haplotype, and to examine the potential genetic contribution of STAT1. To accomplish these aims, we carried out a comprehensive association analysis of 52 tag single nucleotide polymorphisms (SNPs) encompassing the STAT1-STAT4 region.

Methods In the first screening, 52 tag SNPs were selected based on HapMap Phase II JPT (Japanese in Tokyo, Japan) data, and case-control association analysis was carried out on 105 Japanese female patients with SLE and 102 female controls. For associated SNPs, additional cases and controls were genotyped and association was analyzed using 308 SLE patients and 306 controls confirmed a strong association of the rs7574865T allele (SLE patients: 46.3%, controls: 33.5%, \( P = 4.9 \times 10^{-6} \), odds ratio 1.71) as well as TTT haplotype (rs10168266/rs11889341/rs7574865) (\( P = 1.5 \times 10^{-6} \)). The association was stronger in subgroups of SLE with nephritis and anti-double-stranded DNA antibodies. Population attributable risk percentage was estimated to be higher in the Japanese population (40.2%) than in Americans of European descent (19.5%).

Conclusions The same STAT4 risk allele is associated with SLE in Caucasian and Japanese populations. Evidence for a role of STAT1 in genetic susceptibility to SLE was not detected. The contribution of STAT4 for the genetic background of SLE may be greater in the Japanese population than in Americans of European descent.

anti-dsDNA: anti-double-stranded DNA; CI: confidence interval; IFN: interferon; IL: interleukin; IRF5: interferon regulatory factor-5; JPT: Japanese in Tokyo, Japan; LD: linkage disequilibrium; OR: odds ratio; PAR%: population attributable risk percentage; RR: relative risk; SLE: systemic lupus erythematosus; SNP: single nucleotide polymorphism; STAT: signal transducers and activators of transcription.
Introduction

Systemic lupus erythematosus (SLE) is a complex disease characterized by autoantibody production and involvement of multiple organs, including kidneys. Both genetic and environmental factors contribute to the development of SLE [1]. Until now, several genes have been reported to be associated with SLE, of which interferon regulatory factor-5 (IRF5) has been identified as a susceptibility gene common to multiple populations [2-6]. Recently, association of STAT4 (signal transducers and activators of transcription-4) haplotype tagged by rs7574865T with SLE was demonstrated in Caucasians [7]. Subsequently, two genome-wide association studies [8,9], a study focused on the STAT4 region in Caucasians [10], and replication studies in Colombians [11] and a Japanese population [12] have confirmed the association. In addition, an association of STAT4 with SLE phenotypes such as anti-double-stranded DNA (anti-dsDNA) autoantibodies, renal disorder, and age at diagnosis was reported [10,13]. An association of rs7574865 with other autoimmune diseases such as rheumatoid arthritis and primary Sjögren syndrome has also been demonstrated [7,11,12,14]. The STAT4 gene encodes a transcription factor belonging to the STAT family expressed in lymphocytes, macrophages, and dendritic cells. STAT4 is essential for interleukin (IL)-12 signaling and induces interferon-gamma (IFN-γ) production and Th1 differentiation [15]. STAT4 is also activated by type I IFNs (IFN-α/β) [16]. Moreover, the requirement of STAT4 in IL-23-induced IL-17 production has been suggested [17]. Two isoforms of STAT4, STAT4α and STAT4β, are known [18]. Expression of STAT4β, lacking the transactivation domain, did not appear to be affected by the STAT4 single nucleotide polymorphisms (SNPs) [13]. STAT1, another member of the STAT family, is activated by type I IFNs and IFN-γ and plays an important role in immune responses [19]. STAT1 has been reported to be upregulated in peripheral blood mononuclear cells from SLE patients and in kidneys of lupus mice with nephritis [20,21], suggesting that STAT1 may play a role in the pathogenesis of SLE. A possible role of SNPs in the STAT1-STAT4 region other than the haplotype tagged by rs7574865T has recently been excluded in Caucasians [10]. However, in view of substantial differences in disease-associated alleles among populations [2], such analysis should be performed in each population. In this study, we carried out a comprehensive association analysis of the STAT1-STAT4 region with SLE in a Japanese population by scanning 52 tag SNPs of the region encompassing STAT1 and STAT4.

Materials and methods

Patients and healthy controls

Patients and controls were recruited at Juntendo University, the University of Tsukuba, and the University of Tokyo. All patients and healthy controls were unrelated Japanese persons living in the central part of Japan. Three hundred eight SLE patients (18 males and 290 females, average age 41.4 ± 13.5 years) and 306 healthy individuals (119 males and 187 females, average age 32.6 ± 9.8 years) were studied. Diagnosis of SLE and classification of the patients into clinical subsets were carried out according to the American College of Rheumatology criteria for SLE [22]. There was no overlap in cases or controls between this study and the recently reported study in a Japanese population [12]. These studies were reviewed and approved by the research ethics committees of the University of Tsukuba, the University of Tokyo, and Juntendo University. Informed consent was obtained from all study participants.

Association study

Fifty-two tag SNPs in the STAT1-STAT4 region were selected with an r² threshold of 0.9 based on the HapMap Phase II JPT (Japanese in Tokyo, Japan) data. These tag SNPs captured 127 SNPs with a minor allele frequency of greater than or equal to 0.05. First screening was performed in 105 Japanese female SLE patients and 102 female healthy controls using the GoldenGate SNP genotyping assay (Illumina, Inc., San Diego, CA, USA). For the three SNPs that exhibited significant association (P < 0.01), additional samples were genotyped using the TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA), and association was examined in 308 SLE patients and 306 healthy individuals.

Statistical analysis

Association of each SNP was analyzed by chi-square test. Because of the replicative nature of this study, correction for multiple testing was not performed, and unadjusted P values are shown. Haplotype frequency estimation and association analysis using the permutation test were performed with Haploview version 4.0 software (Broad Institute of MIT and Harvard, Cambridge, MA, USA). In the haplotype analysis, the genotype data for rs10168266, rs11889341, and rs7574865 were used and these SNPs were assumed to compose a single haplotype block. In the permutation test, only frequencies of haplotypes in this block were compared (that is, the ‘Haplotypes in Blocks Only’ option was used). Ten million permutations were performed. To test the significance of each SNP conditional on the genotypes of other SNPs, logistic regression analysis was performed under the additive model for the minor allele. Assuming a polymorphic site with two alleles A and a, genotypes were encoded as 0 = aa, 1 = Aa, and 2 = AA. Population attributable risk percentage (PAR%) for the risk genotype (rs7574865T/T and T/G) was estimated by the formula

\[
\text{PAR\%} = \frac{\text{Pe} \times (\text{RR} - 1)}{(\text{Pe} \times (\text{RR} - 1) + 1)},
\]
where Pe represents the risk genotype frequency in the population and RR represents relative risk of the risk genotype [23]. Given the low prevalence of SLE, Pe can be estimated based on the genotype frequencies in healthy controls and RR can be approximated by odds ratio (OR) for the risk genotypes.

**Results and Discussion**

The STAT4 gene is located on 2q32.2-q32.3 adjacent to STAT1 gene, and the region encompassing STAT1 and STAT4 spans approximately 180 kilobase pairs. In the first screening, 52 tag SNPs in the STAT1-STAT4 region, selected with an \( r^2 \) threshold of 0.9 based on the HapMap Phase II JPT data, were genotyped in 105 Japanese female SLE patients and 102 female healthy controls, and allele frequencies were compared between SLE patients and controls. A linkage disequilibrium (LD) plot and the results of the association study in the STAT1-STAT4 region are shown in Figure 1. Pairwise \( r^2 \) values between 52 tag SNPs were calculated using genotyping data from 102 healthy individuals.

Among the tag SNPs, rs10168266C>T, rs11889341C>T, and rs7574865G>T were most significantly associated with SLE in the first screening (\( P < 0.01 \)). Allele frequencies of rs10168266T, rs11889341T, and rs7574865T were significantly increased in SLE compared with healthy controls (Table 1 and Figure 1). These SNPs were located in the introns of STAT4 and in LD with each other. In contrast, significant association was not detected for SNPs in the STAT1 region (\( P > 0.05 \)).

To confirm the association detected in the first screening, additional patients and controls were genotyped for the three SNPs using the TaqMan SNP Genotyping Assay, and association was examined in 308 SLE patients and 306 healthy controls in total (Table 2). Significant deviation from Hardy-Weinberg equilibrium was not detected in healthy controls (\( P > 0.05 \)). The rs7574865T allele, previously shown to be associated with SLE in Caucasians, was significantly increased in SLE patients (46.3%) compared with controls (33.5%, \( P = 4.9 \times 10^{-6}, \text{OR} = 1.71 \)). The association was compatible with the dominant model, under which the OR was 2.19 (T/T + G/T versus G/G).

The SNPs rs11889341 and rs10168266 were in LD with rs7574865 (\( r^2: 0.57 \) to 0.78, \( D^\prime: 0.91 \) to 0.97) and were also significantly associated with SLE (allelic frequency: \( P = 6.6 \times 10^{-6} \) and \( P = 6.3 \times 10^{-6} \), respectively). Haplotype analysis revealed that the haplotype carrying rs10168266T, rs11889341T, and rs7574865T was significantly increased in SLE patients (36.8%, control: 24.3%, \( P = 1.5 \times 10^{-5} \)) whereas the haplotype carrying 10168266C, rs11889341C, and rs7574865G was significantly decreased in SLE (SLE: 52.7%, control: 65.0%, \( P = 1.0 \times 10^{-5} \)). Logistic regression analysis demonstrated that the association of each SNP lost statistical significance when adjusted for genotype of the other SNPs (Table 3). Thus, due to the strong LD, it was impossible to identify a single causative SNP among the three.

We next tested whether STAT4 rs7574865 was associated with phenotypes of SLE such as presence of nephritis, anti-dsDNA antibodies, and early age of onset (less than 20 years) as STAT4 genotype has been shown to be more strongly associated with subgroups of SLE with these phenotypes [10] (Table 4). Association of rs7574865 was observed both in SLE patients with nephritis (\( P = 1.0 \times 10^{-5}, \text{OR} = 1.85 \)) and in those without nephritis (\( P = 0.0031, \text{OR} = 1.55 \)). The association was stronger in SLE patients with nephritis, although the difference between SLE with and without nephritis (case-only analysis) did not reach statistical significance. Similarly, rs7574865T was significantly increased in SLE patients with anti-dsDNA antibodies compared with healthy controls, whereas association was not detected in SLE patients without anti-dsDNA antibodies. The frequency of rs7574865T was slightly higher in the patients with an age of onset of less than

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**Figure 1**

Linkage disequilibrium plot of the STAT1-STAT4 region in a Japanese population and first screening of 52 tag single nucleotide polymorphisms (SNPs). In the upper panel, \( P \) values for differences in allele frequencies were calculated by chi-square test using two-by-two contingency tables. The \(-\log P\) value for each SNP is shown. In the lower panel, \( r^2 \) values calculated using Haplovie version 4.0 software based on data from 102 healthy individuals are shown. The location and direction of transcription of STAT1 and STAT4 are indicated by arrows. SNPs rs10168266, rs11889341, and rs7574865 belong to the same haplotype block.
### Table 1

**Minor allele frequencies and \( P \) values for 52 tag single nucleotide polymorphisms in the STAT1-STAT4 region in the first screening**

| SNP     | Chromosomal position | Minor allele | SLE patients (n = 105) | Controls (n = 102) | \( P \) value |
|---------|----------------------|--------------|------------------------|-------------------|--------------|
| rs3771300 | 191543841            | C            | 0.305                  | 0.309             | 0.929        |
| rs7575823 | 191544163            | A            | 0.167                  | 0.147             | 0.584        |
| rs16824035 | 191545879            | A            | 0.057                  | 0.074             | 0.500        |
| rs1914408 | 191548221            | A            | 0.271                  | 0.314             | 0.344        |
| rs2066804 | 191550004            | A            | 0.471                  | 0.480             | 0.855        |
| rs2280235 | 191552075            | A            | 0.486                  | 0.471             | 0.758        |
| rs3755312 | 191554236            | C            | 0.181                  | 0.176             | 0.905        |
| rs2280234 | 191556344            | G            | 0.162                  | 0.186             | 0.513        |
| rs2280232 | 191559011            | C            | 0.143                  | 0.123             | 0.543        |
| rs11887698 | 191563119            | G            | 0.327                  | 0.304             | 0.629        |
| rs7562024 | 191563766            | G            | 0.090                  | 0.108             | 0.554        |
| rs11904548 | 191567235            | A            | 0.162                  | 0.137             | 0.482        |
| rs12693591 | 191568747            | A            | 0.257                  | 0.235             | 0.606        |
| rs16833155 | 191569622            | A            | 0.043                  | 0.054             | 0.600        |
| rs2066805 | 191571146            | G            | 0.038                  | 0.054             | 0.442        |
| rs11677408 | 191574860            | A            | 0.129                  | 0.108             | 0.514        |
| rs2030171 | 191577408            | G            | 0.329                  | 0.309             | 0.666        |
| rs11693463 | 191578156            | G            | 0.195                  | 0.196             | 0.983        |
| rs11885069 | 191578869            | A            | 0.162                  | 0.137             | 0.482        |
| rs10199181 | 191581798            | T            | 0.267                  | 0.265             | 0.964        |
| rs2066802 | 191582912            | G            | 0.257                  | 0.255             | 0.956        |
| rs13029532 | 191584146            | C            | 0.082                  | 0.103             | 0.457        |
| rs3024904 | 191603447            | A            | 0.112                  | 0.141             | 0.400        |
| rs3024936 | 191603621            | C            | 0.024                  | 0.055             | 0.112        |
| rs1517351 | 191604290            | C            | 0.490                  | 0.464             | 0.602        |
| rs3024896 | 191604961            | A            | 0.448                  | 0.412             | 0.461        |
| rs925847 | 191605785            | A            | 0.538                  | 0.490             | 0.330        |
| rs3024886 | 191606694            | A            | 0.457                  | 0.417             | 0.407        |
| rs6715106 | 191621279            | G            | 0.067                  | 0.083             | 0.520        |
| rs16833215 | 191622044            | G            | 0.495                  | 0.441             | 0.270        |
| rs1400654 | 191623918            | T            | 0.066                  | 0.083             | 0.524        |
| rs3024861 | 191632851            | T            | 0.471                  | 0.397             | 0.127        |
| rs1517352 | 191639709            | A            | 0.481                  | 0.397             | 0.086        |
| rs10168266 | 191644049            | A            | 0.400                  | 0.245             | \(7.6 \times 10^{-4}\) |
| rs7594501 | 191646845            | A            | 0.114                  | 0.152             | 0.250        |
| rs16833239 | 191648050            | A            | 0.110                  | 0.152             | 0.200        |
| rs7601754 | 191648696            | G            | 0.129                  | 0.178             | 0.162        |
20 years as compared with greater than or equal to 20 years, although the difference was not statistically significant. These tendencies are consistent with those reported in Caucasians [10]. These interpretations were not affected when the significance level was corrected for the number of comparisons (three phenotypes).

To evaluate the epidemiological significance of STAT4 polymorphism in the genetic background of SLE in the Japanese population, we estimated the PAR% in Japanese persons and Caucasians using our present data and previously reported data [8,11,12] (Table 5). Because the frequency and OR of the risk genotype of rs7574865 were greater in the Japanese population than those of North Americans of European descent [8], PAR% in the Japanese population (40.2%) was much higher than that of the latter (19.5%). A similarly high PAR% was observed in two of the three Japanese case-control series reported by Kobayashi and colleagues [12] and in Colombians [11]. Because PAR% may be affected by the difference in the method of ascertainment of each study, this comparison may not be completely valid. Nevertheless, these observations suggested that the contribution of STAT4 for SLE is greater in the Japanese population as compared with the Americans of European descent.

At this point, molecular mechanisms that account for the association of STAT4 intron SNPs with SLE remains unclear. Studies with lupus model mice lacking Stat4 showed conflicting results. Stat4 deficiency reduced nephritis and autoantibody production in B6.NZM.Sle1.Sle2.Sle3 mice [24]. In contrast, Stat4-deficient NZM (New Zealand mixed) mice developed accelerated nephritis and increased mortality in the absence of high levels of autoantibodies [25]. STAT4 has been shown to be involved in the induction of IFNγ, differentiation of Th1 and Th17 cells, and signal transduction from type I IFN receptors [15]. Th1 cytokines, especially IFNγ, have been shown to play a role in the pathogenesis of lupus nephritis [26]. Recently, T cells from SLE patients were shown to produce excessive amounts of IFNγ upon stimulation [27]. These observations may implicate the role of STAT4 SNPs in IFNγ production.

The role of type I IFNs in SLE has been established [1]. Elevated serum type I IFN levels and expression of IFN-inducible genes in peripheral mononuclear cells were reported in SLE [28,29]. The association of IRF5, which induces type I IFNs, with SLE has been established [2-6]. STAT4 is activated by type I IFN as well as IL-12 signals and produces IFNγ [15]. Thus, STAT4 may also contribute to SLE as a component of the type I IFN signal pathway. Furthermore, STAT4 has been reported to transduce IL-12 signals to induce IFNγ production in B cells [30].

It is interesting to note that significant association of STAT4 was not observed in SLE patients without anti-dsDNA antibodies (Table 4). It would have been interesting to examine the effect of the genotype on the levels, rather than presence or absence, of anti-dsDNA antibody. However, because the antibody levels fluctuate in association with disease activity and treatment, association with the genotype should be examined.

| rs   | Chromosomal position | Minor allele | OR    | P value |
|------|----------------------|--------------|-------|---------|
| rs11889341 | 191651987 A | 0.443 | 0.299 | 0.003 |
| rs16833249 | 191656517 G | 0.567 | 0.480 | 0.079 |
| rs6434435  | 191662109 A | 0.099 | 0.141 | 0.192 |
| rs7574865  | 191672878 A | 0.471 | 0.324 | 0.002 |
| rs12463658 | 191673589 C | 0.581 | 0.471 | 0.025 |
| rs6752770  | 191681808 G | 0.205 | 0.245 | 0.326 |
| rs1551443  | 191704763 A | 0.238 | 0.206 | 0.431 |
| rs2356350  | 191710783 G | 0.510 | 0.407 | 0.036 |
| rs10189819 | 191716994 G | 0.133 | 0.118 | 0.630 |
| rs7596818  | 191717555 A | 0.320 | 0.295 | 0.580 |
| rs11685878 | 191717700 A | 0.429 | 0.431 | 0.954 |
| rs12991409 | 191717762 G | 0.100 | 0.113 | 0.674 |
| rs12327969 | 191719016 G | 0.390 | 0.402 | 0.811 |
| rs12988825 | 191722509 C | 0.119 | 0.132 | 0.683 |
| rs7572482  | 191723317 G | 0.490 | 0.461 | 0.545 |

*Chromosomal positions are shown according to the National Center for Biotechnology Information (Bethesda, MD, USA) reference assembly. SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; STAT, signal transducers and activators of transcription.
Table 2

Association of STAT4 single nucleotide polymorphisms rs10168266, rs11889341, and rs7574865 with systemic lupus erythematosus

| SNP         | SLE patients (n = 308) | Healthy controls (n = 306) | P value | Odds ratio | 95% CI |
|-------------|------------------------|---------------------------|---------|------------|--------|
|             | Number | Percentage | Number | Percentage |
| rs10168266  |         |           |        |            |        |
| Genotype frequency |       |          |        |            |        |
| C/C         | 118    | 38.3      | 166    | 54.2       |        |
| C/T         | 147    | 47.7      | 122    | 39.9       | 7.5 × 10⁻⁵⁺ | 1.91   | 1.39–2.63⁺ |
| T/T         | 43     | 14.0      | 18     | 5.9        | 4.9 × 10⁻⁵⁺ | 1.75   | 1.37–2.23 |
| Allele frequency |       |          |        |            |        |
| T           | 233    | 37.8      | 158    | 25.8       | 6.3 × 10⁻⁶ | 1.75   | 1.37–2.23 |
| rs11889341  |         |           |        |            |        |
| Genotype frequency |       |          |        |            |        |
| C/C         | 99     | 32.1      | 153    | 50.0       | 6.9 × 10⁻⁶⁺ | 2.11   | 1.52–2.92⁺ |
| C/T         | 161    | 52.3      | 126    | 41.2       | 6.3 × 10⁻⁶⁺ | 2.11   | 1.52–2.92⁺ |
| T/T         | 48     | 15.6      | 27     | 8.8        | 6.6 × 10⁻⁶⁺ | 1.72   | 1.36–2.17 |
| Allele frequency |       |          |        |            |        |
| T           | 257    | 41.7      | 180    | 29.4       | 6.6 × 10⁻⁶⁺ | 1.72   | 1.36–2.17 |
| rs7574865   |         |           |        |            |        |
| Genotype frequency |       |          |        |            |        |
| G/G         | 80     | 26.0      | 133    | 43.5       | 5.3 × 10⁻⁶⁺ | 2.19   | 1.56–3.07⁺ |
| G/T         | 171    | 55.5      | 141    | 46.1       | 5.3 × 10⁻⁶⁺ | 2.19   | 1.56–3.07⁺ |
| T/T         | 57     | 18.5      | 32     | 10.5       | 1.7 × 10⁻⁵⁺ | 1.71   | 1.36–2.15 |
| Allele frequency |       |          |        |            |        |
| T           | 285    | 46.3      | 205    | 33.5       | 4.9 × 10⁻⁶⁺ | 1.71   | 1.36–2.15 |
| rs10168266/rs11889341/rs7574865 |       |          |        |            |        |
| Haplotype frequency |     |          |        |            |        |
| CCG         | 52.7   | 65.0      | 1.0 × 10⁻⁴⁺ | NS⁺       |
| TTT         | 36.8   | 24.3      | 1.5 × 10⁻⁴⁺ | NS⁺       |
| CCT         | 4.9    | 5.1       | NS⁺    |             |
| CTT         | 4.6    | 4.1       | NS⁺    |             |

⁺P values, odds ratios, and 95% confidence intervals (CIs) were calculated under the dominant model for the minor allele. ⁺P values were calculated by permutation test using Haploview version 4.0 software. Ten million permutations were performed. NS, not significant; SLE, systemic lupus erythematosus; STAT, signal transducers and activators of transcription.

Table 3

Logistic regression analysis of the systemic lupus erythematosus-associated single nucleotide polymorphisms in STAT4

| SNP         | P value | rs10168266 | rs11889341 | rs7574865 | P adjusted for |
|-------------|---------|------------|------------|-----------|----------------|
| rs10168266  | 4.9 × 10⁻⁶ | NA         | 0.272      | 0.146     |
| rs11889341  | 4.7 × 10⁻⁶ | 0.251      | NA         | 0.388     |
| rs7574865   | 2.1 × 10⁻⁶ | 0.052      | 0.130      | NA        |

NA, not applicable; SNP, single nucleotide polymorphism; STAT, signal transducers and activators of transcription.
using the lifetime highest anti-dsDNA antibody level of each patient. Such data were not available for this study, and we hope that we can address this issue in the future.

Most of these observations imply that STAT4 risk genotype may be associated with an elevated expression level and/or function of STAT4 protein. A recent study reported that the STAT4 risk allele was associated with overexpression of STAT4 in osteoblasts but not in B cells [13]. To address the significance of such findings, it will be necessary to examine the effect of this genotype on the expression levels and splicing isoforms in T and B cells.

**Conclusion**

Through comprehensive association analysis of the STAT1-STAT4 region with SLE in the Japanese population, we demonstrated that the same STAT4 risk allele in Caucasians was strongly associated with susceptibility to SLE in the Japanese population. In contrast, evidence for an association of STAT1 SNPs was not observed. The contribution of STAT4 SNPs to

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**Table 4**

| Association of STAT4 rs7574865 with characteristics of systemic lupus erythematosus such as nephritis, age of onset, and anti-double-stranded-DNA antibodies |
|--------------------------------------------------|-----------------|-----------------|---------------|
| T allele                                         | P value         | Odds ratio (95% CI) |
| Number   | Frequency        |                |               |
| Case subgroup versus healthy controls           |                 |                 |
| Nephritis                                       |                 |                 |
| Present (n = 165)                               | 159             | 48.2%           | 1.0 × 10⁻⁵      | 1.85 (1.41–2.42) |
| Absent (n = 138)                                | 121             | 43.8%           | 0.0031         | 1.55 (1.16–2.07) |
| Anti-double-stranded DNA antibodies              |                 |                 |
| Present (n = 130)                               | 125             | 48.1%           | 4.9 × 10⁻⁵      | 1.84 (1.37–2.47) |
| Absent (n = 34)                                 | 24              | 35.3%           | NS             | 1.08 (0.64–1.83) |
| Age of onset                                    |                 |                 |
| <20 years (n = 86)                              | 83              | 48.3%           | 3.9 × 10⁻⁴      | 1.85 (1.32–2.60) |
| ≥20 years (n = 198)                             | 180             | 45.5%           | 1.4 × 10⁻⁴      | 1.65 (1.28–2.14) |
| Healthy controls (n = 306)                      | 205             | 33.5%           |                |                 |
| Case-only (present versus absent or <20 versus ≥ 20 years) |     |                 |
| Nephritis                                       |                 |                 |
| NS                                              |                 | 1.19 (0.86–1.64) |
| Anti-double-stranded DNA antibodies              |                 |                 |
| NS                                              |                 | 1.70 (0.98–2.95) |
| Age of onset                                    |                 |                 |
| NS                                              |                 | 1.12 (0.78–1.60) |

Systemic lupus erythematosus (SLE) patients were stratified into subgroups according to the presence or absence of nephritis, anti-double-stranded DNA (anti-dsDNA) antibodies, and age of onset (<20 or ≥ 20 years). Allele frequencies were compared between each SLE subgroup and healthy controls as well as between SLE subgroups (case-only analysis, nephritis present versus absent, anti-dsDNA antibodies present versus absent, and age of onset <20 versus ≥ 20 years). CI, confidence interval; NS, not significant; STAT, signal transducers and activators of transcription.

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**Table 5**

| Population attributable risk percentage of STAT4 rs7574865 under the dominant model |
|-------------------------------------------------------------------------------------|
| Population [reference] | Frequency of (T/T+T/G) | Odds ratio | PAR%  |
|------------------------|------------------------|------------|-------|
| Japanese (this study)  | 56.5%                  | 2.19       | 40.2% |
| Japanese (TWMU) [12]   | 52.3%                  | 1.81       | 29.7% |
| Japanese (RIKEN) [12]  | 51.7%                  | 1.51       | 20.8% |
| Japanese (Tokushima/Fukuoka) [12] | 51.9% | 2.07       | 35.8% |
| Americans of European descent [8]       | 41.2%                  | 1.59       | 19.5% |
| Colombians [11]        | 51.7%                  | 1.87       | 31.0% |

PAR%, population attributable risk percentage; RIKEN, The Institute of Physical and Chemical Research, Wako, Japan; STAT, signal transducers and activators of transcription; TWMU, Tokyo Women’s Medical University, Tokyo, Japan.
the genetic background of SLE may be greater in the Japanese population than in Americans of European descent.

**Competing interests**

RRG, GH, and TWB are employees of and hold stocks or shares in Genentech, Inc. (South San Francisco, CA, USA). The other authors declare that they have no competing interests.

**Authors’ contributions**

AK participated in the study design, carried out all genotyping and statistical analyses, and wrote the manuscript. II, KH, MK, and TA participated in the first screening using Illumina GoldenGate assay (with AK), including tag SNP selection, genotyping, and statistical analysis. JO carried out statistical analysis with AK and helped in the manuscript preparation. TH, DG, IM, SI, AT, YT, HH, and TS recruited Japanese patients with SLE and collected clinical information. RRG and GH provided Caucasian data. NT conceived of the study, together with TWB, and participated in its design and coordination, recruited patients and controls, and helped in the manuscript preparation. All authors read and approved the final manuscript.

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**References**

1. Kyogoku C, Tsujiya N: A compass that points to lupus: genetic studies on type 1 interferon pathway. *Genes Immun* 2007, 8:445-459.

2. Kawasaki A, Kyogoku C, Ohashi J, Miyashita R, Hikami K, Kusaoi M, Takasaki Y, Hashimoto H, Behrens TW, Tsujiya N: Association of IRF5 polymorphisms with systemic lupus erythematosus in a Japanese population: support for a crucial role of intron 1 polymorphisms. *Arthritis Rheum* 2008, 58:926-934.

3. Sigurdsson S, Nordmark G, Göring HH, Lindroos K, Wiman AC, Kallenberg CG, Bijl M, Skopouli FN, Mavromati M, Migliaresi S, Balada E, Carreira P, Gomez-Reino JJ, Gonzalez A: Specificity of the STAT4 association for severe disease manifestations of systemic lupus erythematosus. *PLoS Genet* 2008, 4:e1000084.

4. Sigurdsson D, Nordmark G, Garnier S, Grundberg E, Hamada D, Yasui N, Inoue H, Itakura M, Okamoto H, Kamatani N, Momohara S: Association of STAT4 with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in the Japanese population. *Arthritis Rheum* 2008, 59:1940-1946.

5. Sigurdsson S, Nordmark G, Garnier S, Grundberg E, Kwan T, Nils-son O, Eloranta M-L, Gunnarsson I, Svenungsson E, Sturfelt G, Bengtsson AA, Rantapää-Dahlqvist S, Petri M, Manzi S, Seldin MF, Gregersen PK, Behrens TW, Criswell LA: Specificity of the STAT4 risk haplotype for systemic lupus erythematosus is overexpressed, correlates with anti-dsDNA production and shows additive effects with two IRF5 risk alleles. *Hum Mol Genet* 2008, 17:2869-2878.

6. Kornman BD, Alba MI, Lee JM, Alevizos I, Smith JA, Nikolov NP, Kastner DL, Remmers EF, Illlei GG: Variant form of STAT4 is associated with primary Sjögren’s syndrome. *Genes Immun* 2008, 9:267-270.

7. Watford WT, Hisong BD, Bream JH, Kanno Y, Muul L, O’Shea JJ: Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev* 2004, 202:139-156.

8. Nguyen KB, Watford WT, Salomon R, Hofmann SR, Pien GC, Mominobu A, Gadina M, O’Sheaj J, Biron CA: Critical role for STAT4 activation by type 1 interferons in the interferon-gamma response to viral infection. *Science* 2002, 297:2063-2066.

9. Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, O’Malley JT, Kapur R, Levy DE, Kansas GS, Kaplan MH, Stat3 and Stat4 direct development of IL-17-secreting Th cells. *J Immunol* 2007, 178:4901-4907.

10. Hoey T, Zhang S, Schwartz M, Yu Q, Ramchandani S, Xu X, Naegker LK, Sun YL, Kaplan MH: Distinct requirements for the naturally occurring splice forms Stat3α and Stat3β in IL-12 responses. *EMBO J* 2003, 22:4237-4248.
19. Takeda K, Akira S: STAT family of transcription factors in cytokine-mediated biological responses. Cytokine Growth Factor Rev 2000, 11:199-207.
20. Baechler EC, Gregersen PK, Behrens TW: The emerging role of interferon in human systemic lupus erythematosus. Curr Opin Immunol 2004, 16:801-807.
21. Dong J, Wang QX, Zhou CY, Ma XF, Zhang YC: Activation of the STAT1 signalling pathway in lupus nephritis in MRL/lpr mice. Lupus 2007, 16:101-109.
22. Hochberg MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997, 40:1725.
23. SchilDKraut JM: Examining complex genetic interactions. In Approach to Gene Mapping in Complex Human Diseases Edited by: Haines JL, Pericak-Vance MA. New York, NY: Wiley-Liss; 1998:379-410.
24. Xu Z, Duan B, Croker BP, Morel L: STAT4 deficiency reduces autoantibody production and glomerulonephritis in a mouse model of lupus. Clin Immunol 2006, 120:189-198.
25. Jacob CO, Zang S, Li L, Giobanu V, Quismorio F, Mizutani A, Satoh M, Koss M: Pivotal role of Stat4 and Stat6 in the pathogenesis of the lupus-like disease in the New Zealand mixed 2328 mice. J Immunol 2003, 171:1564-1571.
26. Akahoshi M, Nakashima H, Tanaka Y, Kohsaka T, Nagano S, Ohgami E, Arinobu Y, Yamaoka K, Niire H, Shinozaki M, Hirakata H, Honuchi T, Otsuka T, Nihon Y: Th1/Th2 balance of peripheral T helper cells in systemic lupus erythematosus. Arthritis Rheum 1999, 42:1644-1648.
27. Harigai M, Kawamoto M, Hara M, Kubota T, Kamatani N, Miyasaka N: Excessive production of IFN-γ in patients with systemic lupus erythematosus and its contribution to induction of B lymphocyte stimulator/B cell-activating factor/TNF ligand superfamily-13B. J Immunol 2008, 181:2211-2219.
28. Crow MK: Interferon-α: a new target for therapy in systemic lupus erythematosus? Arthritis Rheum 2003, 48:2396-2401.
29. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espindola J, Shark KB, Grande WJ, Hughes KM, Kapur V, Gregersen PK, Behrens TW: Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci USA 2003, 100:2610-2615.
30. Burali D, de Goër de Herve M-G, Giron-Michel J, Azzarone B, Delfraissy J-F, Taoufik Y: In human B cells, IL-12 triggers a cascade of molecular events similar to Th1 commitment. Blood 2003, 102:4084-4089.