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ORIGINAL ARTICLE

# Primary and secondary surveys on epidemiology of Sjögren's syndrome in Japan

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**Abstract**

*Objective.* To characterize the epidemiology of Sjögren's syndrome (SS), including prevalence, disease type, extra-glandular involvement, satisfaction of diagnostic criteria sets, and treatment used in Japan.

*Methods.* The Research Team for Autoimmune Diseases, the Research Program for Intractable Disease by the Ministry of Health, Labor and Welfare conducted primary and secondary surveys on epidemiology of SS in 2011. The primary survey covered 4,729 out of 14,095 Japan-wide Hospital Departments to investigate the prevalence of SS. The secondary survey encompassed 214 Hospital Departments that agreed to the survey, to characterize disease type, extra-glandular involvement, satisfaction of diagnostic criteria sets, and treatments.

*Results.* The number of patients with SS in Japan estimated by the primary survey was 68,483. The secondary survey involving data collected from 2,195 SS patients from 98 Hospital Departments showed that the mean age of patients was 60.8 ± 15.2 years, male/female ratio was 1/17.4, primary/secondary SS was about 60%/40% and glandular/extra-glandular form in primary SS was about 70%/25%. The satisfaction rate was 53.8% for the 1999 revised Japanese Ministry of Health criteria for the diagnosis of SS, 47.7% for the 2002 American–European Consensus Group classification criteria for SS and 49.6% for 2012 American College of Rheumatology classification criteria for SS. Corticosteroids were used by 752 of 2,195 patients (34%), immunosuppressants by 358 patients (16%), biologics by 68 patients (3%) and secretagogues by 695 patients (32%).

*Conclusion.* The surveys provided valuable information on the epidemiology of SS including prevalence, disease type, extra-glandular involvement, satisfaction of diagnostic criteria sets and treatments used today in Japan.

**Keywords**

Criteria, Epidemiology, Extra-glandular form, Glandular form, Sjögren's syndrome, Treatment

**History**

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## Introduction

Sjögren's syndrome (SS) is an autoimmune disease that affects exocrine glands including salivary and lacrimal glands. It is characterized pathologically by lymphocytic infiltration into the exocrine glands, and clinically by dry mouth and dry eyes. SS is subcategorized into primary SS, which is not associated with any other well-defined connective tissue disease (CTD), and secondary SS, which is associated with other well-defined CTD [1]. Primary SS is further subdivided into the glandular form, with involvement of the exocrine glands only, and the extra-glandular form, with involvement of organs other than exocrine glands.

SS is a common autoimmune disease, and patients with SS present with a variety of clinical symptoms and signs other than dry mouth and dry eyes, such as general fatigue, arthralgia, myalgia, gastrointestinal symptoms, dysesthesia and depression. Accordingly, patients with SS might consult not only rheumatologists,

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dentists and ophthalmologists, but also general physicians, otolaryngologists, orthopedists, gastroenterologists, neurologists and psychiatrists. Several criteria sets have been proposed for the diagnosis of SS. Indeed, the revised criteria for the diagnosis of SS issued by the Japanese Ministry of Health (JPN) (1999) [2], as well as the American–European Consensus Group classification criteria for SS (AECG) (2002) [1], are usually used both in daily clinical practice and in clinical studies in Japan [3]. In addition to these two sets of diagnostic criteria, the American College of Rheumatology (ACR) recently published the ACR classification criteria for SS (2012), which were proposed by the Sjögren's International Collaborative Clinical Alliance (SICCA) [4].

The complex nature of SS makes it difficult to assess the precise epidemiology of SS [5]. Actually, the reported prevalence of SS varies widely among different studies, ranging from 0.1% to 4.8% [5]. A few studies have also estimated the population prevalence of SS in Japan. In 1993, The Research Team for Autoimmune Diseases and Epidemiology of the Japanese Ministry of Health estimates the number of patients with SS at 17000 [6]. In a more recent study conducted in 2008, the prevalence of SS defined by AECG criteria among Nagasaki atomic bomb survivors was 2.3% (23/1008) [7]. However, the exact incidence of SS throughout Japan is currently unknown.

To characterize the epidemiology of SS, including prevalence, disease type, extra-glandular involvement, satisfaction of criteria and treatment modalities used in Japan, The Research Team for Autoimmune Diseases, the Research Program for Intractable Disease by the Ministry of Health, Labor and Welfare (MHLW) conducted the primary and secondary survey on epidemiology of SS in 2011.

## Methods

### Primary survey

The Research Team for Autoimmune Diseases, the Research Program for Intractable Disease by MHLW conducted a primary survey on the epidemiology of SS in 2011. We identified the target departments of hospitals using the following protocol. We identified 7,999 Departments of Internal Medicine, 2,391 Departments of Ophthalmology, 2,011 Departments of Otolaryngology, 936 Departments of Rheumatology and 758 Departments of Oral Surgery (total 14,095 Hospital Departments) across Japan. These departments were divided into seven categories according to the number of beds in each hospital, including $< 100$, 100–199, 200–299, 300–399, 400–499, and $\geq 500$ beds, and university hospitals (Supplementary material, Table 1 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765). We selected the target departments at random by the following extractability, 5% for hospitals with $< 100$ beds, 10% for hospitals with 100–199 beds, 20% for 200–299 beds,

Table 1. Reported patients in primary survey.

| | Category | Target departments | Response (A) | Response rate (%) | Reported patients (B) | Total departments (C) | Total patients (D) |
|---|---|---|---|---|---|---|---|
| Internal Medicine | University hospital | 147 | 40 | 27 | 1077 | 147 | 3958 |
| | 500 beds and over | 384 | 69 | 18 | 1827 | 384 | 10168 |
| | 400–499 beds | 256 | 43 | 17 | 132 | 321 | 985 |
| | 300–399 beds | 265 | 46 | 17 | 195 | 662 | 2806 |
| | 200–299 beds | 204 | 43 | 21 | 237 | 1023 | 5638 |
| | 100–199 beds | 236 | 61 | 26 | 189 | 2367 | 7334 |
| | Under 100 beds | 158 | 36 | 23 | 26 | 3095 | 2235 |
| | Total | 1650 | 338 | 20 | 3683 | 7999 | 33125 |
| Ophthalmology | University hospital | 129 | 28 | 22 | 1544 | 129 | 7113 |
| | 500 beds and over | 286 | 52 | 18 | 354 | 286 | 1947 |
| | 400–499 beds | 169 | 37 | 22 | 143 | 211 | 815 |
| | 300–399 beds | 153 | 39 | 25 | 62 | 387 | 615 |
| | 200–299 beds | 78 | 9 | 12 | 12 | 391 | 521 |
| | 100–199 beds | 59 | 5 | 8 | 4 | 582 | 466 |
| | Under 100 beds | 19 | 2 | 11 | 3 | 405 | 405 |
| | Total | 893 | 172 | 19 | 2122 | 2391 | 11883 |
| Otolaryngology | University hospital | 127 | 44 | 35 | 199 | 127 | 574 |
| | 500 beds and over | 282 | 71 | 25 | 283 | 282 | 1124 |
| | 400–499 beds | 160 | 39 | 24 | 30 | 204 | 157 |
| | 300–399 beds | 148 | 30 | 20 | 70 | 369 | 123 |
| | 200–299 beds | 71 | 18 | 25 | 19 | 355 | 375 |
| | 100–199 beds | 44 | 17 | 39 | 0 | 446 | 0 |
| | Under 100 beds | 11 | 3 | 27 | 0 | 228 | 0 |
| | Total | 843 | 222 | 26 | 601 | 2011 | 2353 |
| Rheumatology | University hospital | 48 | 16 | 33 | 3045 | 48 | 9135 |
| | 500 beds and over | 67 | 16 | 24 | 762 | 67 | 3191 |
| | 400–499 beds | 48 | 11 | 23 | 100 | 48 | 436 |
| | 300–399 beds | 67 | 12 | 18 | 220 | 67 | 1228 |
| | 200–299 beds | 130 | 30 | 23 | 370 | 130 | 1603 |
| | 100–199 beds | 270 | 54 | 20 | 102 | 270 | 510 |
| | Under 100 beds | 306 | 68 | 22 | 170 | 306 | 765 |
| | Total | 936 | 207 | 22 | 4769 | 936 | 16869 |
| Oral surgery | University hospital | 83 | 38 | 46 | 578 | 83 | 1262 |
| | 500 beds and over | 167 | 53 | 32 | 504 | 167 | 1588 |
| | 400–499 beds | 71 | 25 | 35 | 33 | 87 | 115 |
| | 300–399 beds | 50 | 16 | 32 | 47 | 125 | 367 |
| | 200–299 beds | 20 | 8 | 40 | 62 | 101 | 783 |
| | 100–199 beds | 13 | 4 | 31 | 0 | 126 | 0 |
| | Under 100 beds | 3 | 1 | 33 | 2 | 69 | 138 |
| | Total | 407 | 145 | 36 | 1226 | 758 | 4253 |
| Total | | 4729 | 1084 | 23 | 12401 | 14095 | 68483 |

Calculation of total patients (D); Sum of [Reported patients (B)/Response (A) X Total departments (C) in each category].

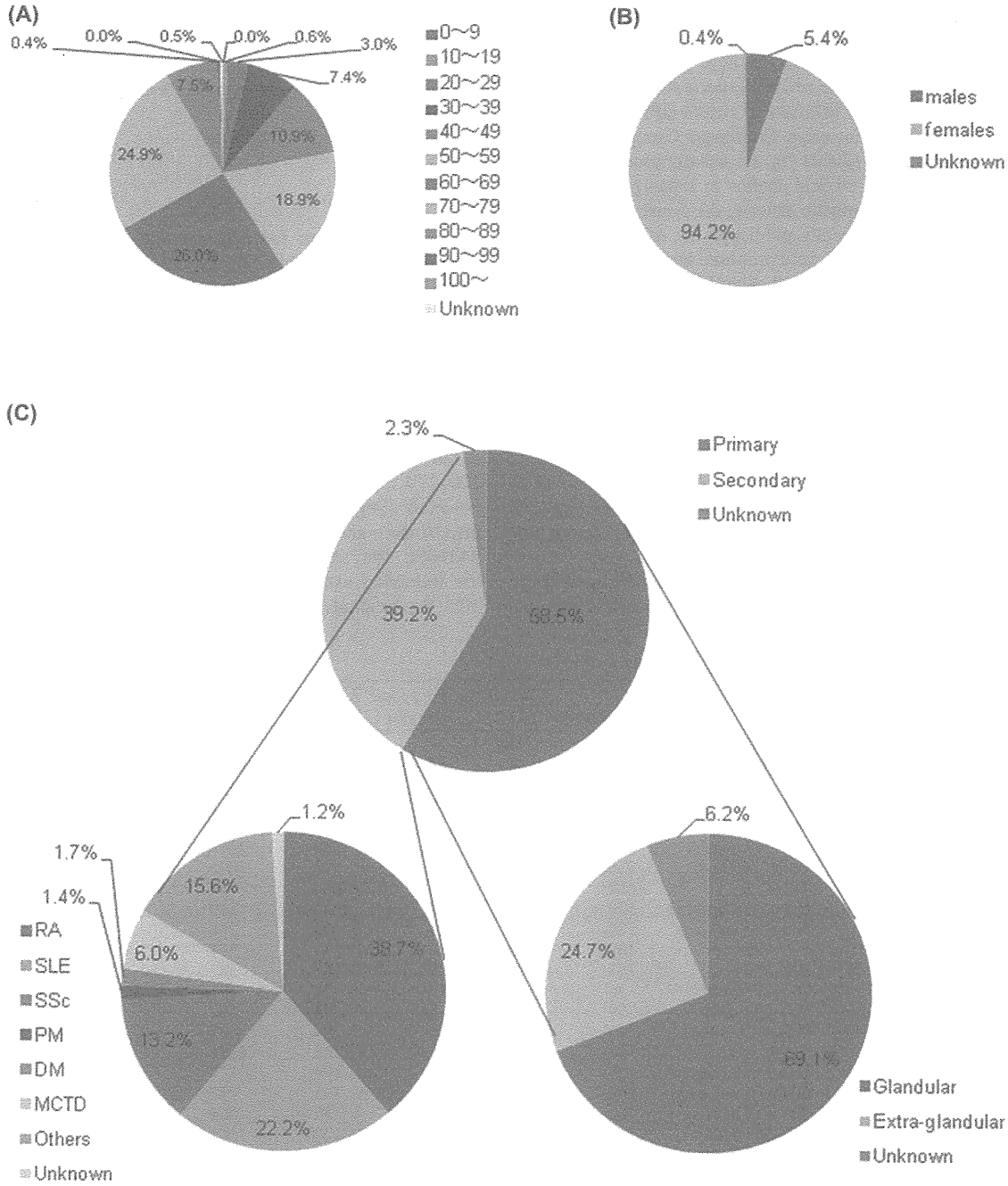


Figure 1. Characteristics of 2,195 patients with SS who participated in the secondary survey. (A). Age distribution; (B). Gender; (C). Disease type. *Top:* frequency of primary and secondary SS. *Bottom right:* frequency of glandular and extra-glandular forms among patients with primary SS. *Bottom left:* frequency of other CTDs in patients with secondary SS. RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; PM: polymyositis; DM: dermatomyositis; MCTD: mixed CTD.

40% for 300–399 beds, 80% for 400–499 beds and 100% for ≥500 beds, university hospitals, and Departments of Rheumatology (Supplementary material, Table 1 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765). Finally, we selected 1,650 Departments of Internal Medicine, 893 Departments of Ophthalmology, 843 Departments of Otolaryngology, 936 Departments of Rheumatology and 407 Departments of Oral Surgery (total 4,729 Hospital Departments). Thus, the primary survey was conducted in these 4,729 departments out of 14,095 Hospital Departments across Japan (Supplementary material, Table 1 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765). In the primary survey, we determined the number of patients with SS who consulted each department in 2010 (from 1 January to 31 December). Consent to participate in the secondary survey was obtained from each Hospital Department.

### Secondary survey

The Research Team for Autoimmune Diseases also conducted a secondary survey on the epidemiology of SS in 2011. The secondary survey was performed in 214 Hospital Departments that agreed to participate in the survey. In the secondary survey, we investigated the effect of age, sex, disease type, extra-glandular

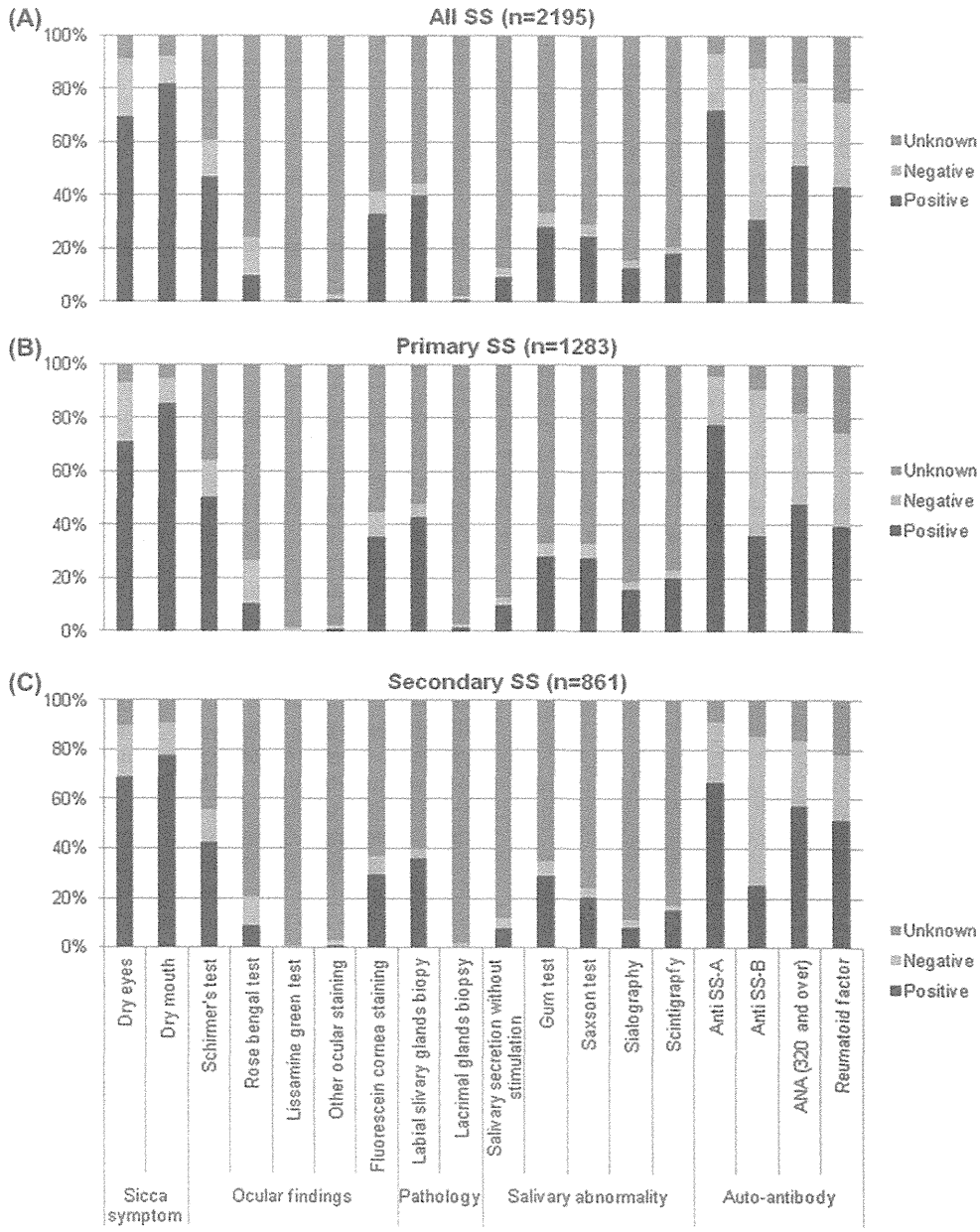


Figure 2. Positivity of various sicca symptoms and objective findings. The histogram shows proportion of patients with positive, negative and unknown (not performed) sicca symptoms and objective findings in (A) all patients with SS, (B) patients with primary SS and (C) patients with secondary SS.



involvements, satisfaction of criteria and treatment of patients with SS in Japan.

## Results

### Primary survey

Responses to the primary survey were received from 1,084 out of 4,729 Hospital Departments (response rate: 23%) (Table 1). The total number of patients with SS across Japan was calculated by the following formula: Sum of [Reported patients (B)/Return (A) X Total departments (C) in each category]. The estimated total number of patients with SS across Japan was 68,483 (Table 1). During the primary survey, 214 Hospital Departments consented to the secondary survey (Supplementary material, Table 2 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765).

### Secondary survey

Responses to the secondary survey were received from 98 out of 214 Hospital Departments (response rate: 45.8%) (Supplementary material, Table 2 available online at http://informahealthcare.com/doi/abs/10.3109/14397595.2013.843765). Data were collected on 2,195 SS patients in the secondary survey. The mean age of the 2,195 SS patients was 60.8 ± 15.2 years, and the age of 69.7% of patients (1,530/2,195 patients) ranged from 50 to 79 years (Figure 1A). Furthermore, 94.2% of the patients were females, with a male/female ratio of 1/17.4 (Figure 1B). Primary SS was diagnosed in 1,283 out of 2,195 patients (58.5%), whereas secondary SS was diagnosed in 861 out of 2,195 patients (39.2%) (Figure 1C). With regard to primary SS, 886 out of 1,283 patients (69.1%) had the glandular form, and 317 out of 1,283 patients (24.7%) had the extra-glandular form (Figure 1C). In patients with secondary SS, rheumatoid arthritis (RA) was diagnosed in 38.7%

(333 out of 861 patients), and systemic lupus erythematosus (SLE) in 22.2% (191 out of 861 patients) (Figure 1C).

Figure 2 shows the proportion of patients with various sicca symptoms and the objective findings included by the diagnostic criteria set. The frequencies of dry eyes and mouth, and anti-SS-A antibody were high, while ocular staining and salivary examinations were not performed in many patients with SS (Figure 2A), primary SS (Figure 2B) and secondary SS (Figure 2C). Histopathological findings of labial salivary glands biopsies were positive in about 40% of patients, but the examination was performed in only about 40% of patients (Figure 2A–C). Interestingly, 597 out of 2,195 SS patients (27.2%) were seronegative SS (both anti-SS-A and SS-B antibodies were negative or unknown).

Figure 3 displays the Venn diagram showing comparison of satisfaction with JPN, AECG and ACR diagnostic criteria set in all SS, primary SS and secondary SS. The satisfaction rate was 53.8% (1,182/2,195) for JPN criteria, 47.7% (1,046/2,195) for AECG criteria, and 49.6% (1,089/2,195) for ACR criteria in all SS patients (Figure 3A). However, 798 out of 2,195 patients did not satisfy any criteria sets (Figure 3A). The satisfaction rate was 61.1% (784/1283) for JPN, 59.2% (760/1283) for AECG and 54.5% (699/1283) for ACR in primary SS patients (Figure 3B). The satisfaction rate was 44.9% (387/861) for JPN, 31.6% (272/861) for AECG and 44.1% (380/861) for ACR in secondary SS patients (Figure 3C). The agreement between the JPN and ACR criteria sets was good (kappa coefficient; 0.77–0.80), compared with moderate agreement between the AECG and the other two criteria sets (kappa coefficient; 0.50–0.56) in the diagnosis of all SS, primary SS and secondary SS patients (Table 2).

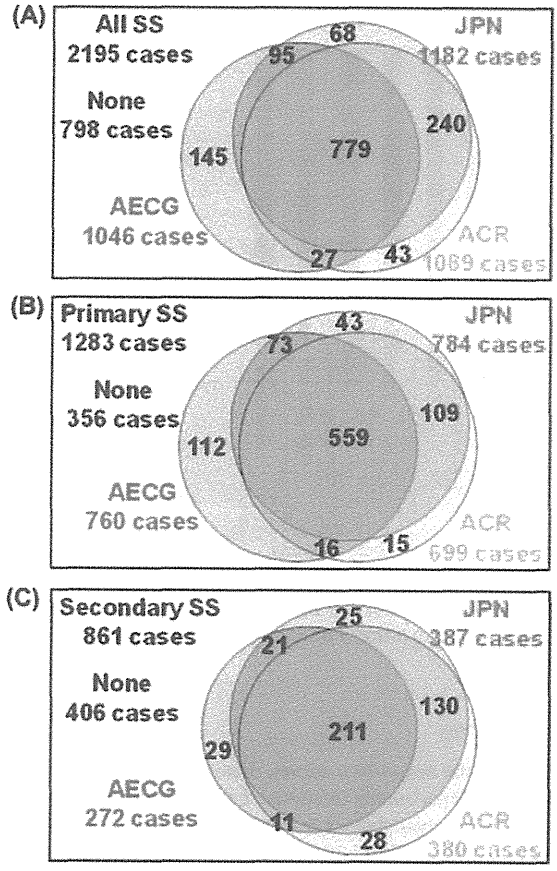


Figure 3. Venn diagrams showing comparison of satisfaction of the three tested criteria sets. (A) Comparison of satisfaction of the three tested criteria sets using data of all (n = 2,195) patients with SS. (B) Comparison of satisfaction of the three tested criteria sets using data of 1,283 patients with primary SS. (C) Comparison of satisfaction of the three tested criteria sets using data of 861 patients with secondary SS. Numbers: number of patients who satisfied each set of criteria, None: patients who did not satisfy the criteria of any of the three systems. JPN: The revised Japanese Ministry of Health criteria for the diagnosis of SS (1999); AECG: The American–European Consensus Group classification criteria for SS (2002); ACR: American College of Rheumatology classification criteria for SS (2012).

Table 2. Agreement between three criteria sets assessed by kappa coefficient.

| | All SS 2195 cases | Primary SS 1283 cases | Secondary SS 861 cases |
|---|---|---|---|
| | | kappa coefficient | |
| JPN vs. AECG | 0.56 | 0.54 | 0.53 |
| JPN vs. ACR | 0.79 | 0.77 | 0.80 |
| AECG vs. ACR | 0.52 | 0.51 | 0.50 |

JPN: The revised Japanese Ministry of Health criteria for the diagnosis of SS (1999).
AECG: The American–European Consensus Group classification criteria for SS (2002).
ACR: American College of Rheumatology classification criteria for SS (2012).

Figures 4 and 5 summarize the types of treatment according to SS disease type. Corticosteroids were administrated in 752/2,195 SS patients (34.3%), in 270/1283 primary SS patients (21.0%) and in 475/861 secondary SS patients (55.2%) (Figure 4A). Among the corticosteroid-treated primary SS group, 126 patients (46.7%) had the glandular form, whereas 132 patients (48.9%) had the extra-glandular form. Immunosuppressants were used in 358/2,195 SS patients (16.3%), in 68/1,283 primary SS patients (5.3%) and in 287/861 secondary SS patients (33.3%) (Figure 4B). Among the immunosuppressants-treated primary SS group, 26 patients (38.2%) had the glandular form, whereas 38 patients (55.9%) had the extra-glandular form. Biologics were administrated in 68/2,195 SS patients (3.1%) (infliximab in 8, etanercept in 21, adalimumab in 10, tocilizumab in 13, abatacept in 10, rituximab in 1, and others and unknown in 5 patients), in 7/1,283 primary SS patients (0.5%) and in 59/861 secondary SS (6.9%) (Figure 5A). Among the biologics-treated secondary SS group, 49 patients (83.1%) had RA (Figure 5A). Secretagogues, such as pilocarpine and cevimeline, were administrated in 695/2,195 SS patients (31.7%), in 470/1,283 primary SS patients (36.6%) and in 212/861 secondary SS patients (24.6%) (Figure 5B).

## Discussion

Although SS is a common autoimmune disease, the precise epidemiology of this disease remains poorly defined [8]. It is important to determine the epidemiology including prevalence, disease type, extra-glandular involvements, satisfaction of diagnostic criteria and treatment modalities for SS, because such information could help establish diagnostic and therapeutic guidelines, as well as validation of criteria sets. For this reason, the Research Team for Autoimmune Diseases, the Research Program for Intractable Disease of MHLW conducted primary and secondary surveys on the epidemiology of SS in Japan.

The study identified several important findings about SS. First, the estimated number of patients with SS in Japan was 68,483. Based on a total population of Japan at October 1, 2011 of 127,799,000, the prevalence of SS in Japan is 0.05%. This rate is lower than the minimum lowest prevalence of SS of 0.1 reported for other countries [5]. What is the reason for the low prevalence of SS in Japan? In many studies on the prevalence of SS performed in other countries, randomly selected population has been targeted, and SS criteria sets such as AECG criteria have been adopted for the diagnosis of SS [5]. On the other hand, in this survey, we targeted patients with SS who consulted their physicians, rather than

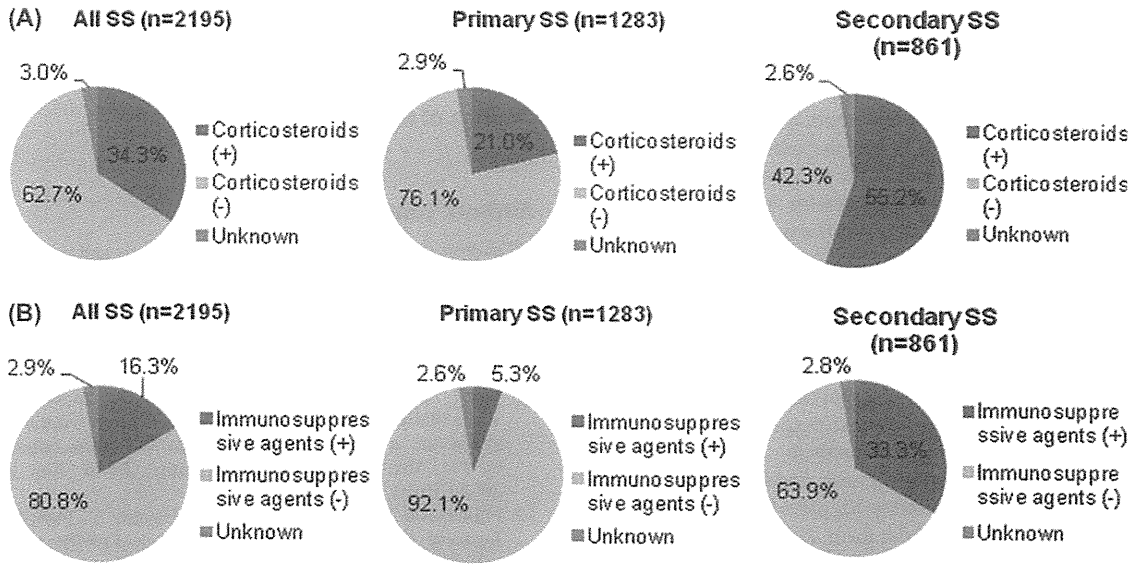


Figure 4. Treatments used in patients with SS and relationship between treatments and disease type (corticosteroids and immunosuppressive agents). (A) Corticosteroids. (B) Immunosuppressive agents. +: patients treated with drugs; -: patients treated without drugs.

the general public, and diagnosis of SS depended on the physician judgment. Thus, the present study underestimates the prevalence of SS in Japan, especially sub-clinical cases might be overlooked.

Second, the study characterized the distribution of age, gender and SS disease type in patients with SS in Japan. Previous reports indicated that SS affects mainly middle-aged females, with a female to male ratio of 9:1 [5]. We confirmed these features of SS in this survey, with the mean age of the group of 60.8 ± 15.2 years, and a female to male ratio of 17.4:1. Previous studies reported that almost half of SS patients develop extra-glandular disease, such as arthralgia/arthritis (> 50%), interstitial nephritis (25%), interstitial lung diseases (30%) and peripheral polyneuropathy (20%) [5,9]. We demonstrated in the present study that the ratio of the primary to secondary SS was about 60% to 40%, with the glandular to extra-glandular form ratio of about 70% to 25% among patients with primary SS. We also confirmed the importance of screening for systemic organ involvement in SS patients.

Third, the satisfaction rate for three sets of criteria, including JPN, AECG and ACR, was only ~50% in this survey. This finding indicates that about half of the SS patients were not diagnosed by the diagnostic criteria but rather by the physician judgment. Importantly, pathological examination of labial salivary glands biopsy was performed only in about 40% of patients, but was not performed in about 60% of patients. This low frequency of labial

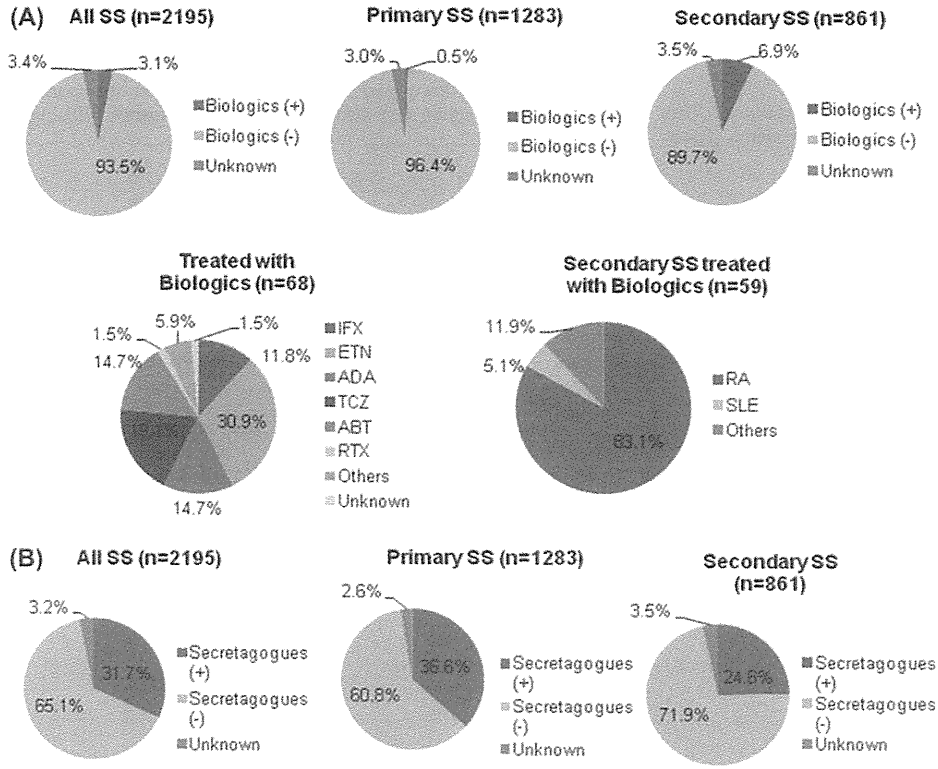


Figure 5. Treatments used in patients with SS and relationship between treatments and disease type (biologics and secretagogues). (A) Biologics. (B) Secretagogues. +, patients treated with drugs; -, patients treated without drugs; IFX, Infliximab; ETN, etanercept; ADA, adalimumab; TCZ, tocilizumab; ABT, abatacept; RTX, rituximab; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

salivary gland biopsy could be problematic, if the ACR criteria are to be adopted in Japan, because ACR criteria comprise only three items: autoantibodies, labial salivary glands biopsy and ocular staining [4]. Moreover, in this survey, the agreement between the JPN and ACR criteria sets was good, compared with moderate agreement between the AECG and the other two criteria sets in the diagnosis of SS. These results are consistent with the previous study performed by the Research Team for Autoimmune Diseases, the Research Program for Intractable Disease by MHLW using data of 694 patients with SS or suspected SS who had been checked for all four criteria of the JPN (pathology, oral, ocular and anti-SS-A/SS-B antibodies) [3].

Finally, this survey also characterized for the first time the treatment modalities applied for SS in Japan. Corticosteroids were used in 34% of patients, immunosuppressants in 16%. As expected, both corticosteroids and immunosuppressive agents were used mainly in patients with secondary SS. Interestingly, about half of primary SS patients treated with corticosteroids had the glandular form of SS, whereas only 38% of primary SS patients treated with immunosuppressants had the glandular form. Although the effectiveness of corticosteroids for glandular involvement of SS has not been established [10], in Japan, 10% of primary SS patients could be treated with corticosteroids for glandular involvement. On the other hand, immunosuppressive agents might be used in primary SS for mainly extra-glandular involvements. Biologics were administrated only in 3% of SS, and the main target of biologics was RA, which was associated with secondary SS. Secretagogues were used in 32% of patients with SS, and a larger proportion of patients with primary SS used these drugs than those with secondary SS. This finding suggests that dryness in primary SS is more severe than that in secondary SS.

Although this survey is cross-sectional, these important findings should be useful for the establishment of diagnostic and therapeutic guidelines for SS. Longitudinal surveys and prospective studies are needed to confirm these results.

In conclusion, the primary and secondary surveys employed in the present study provided valuable information on the epidemiology of SS, including prevalence, disease type, extra-glandular involvement, satisfaction of criteria and treatment used today in Japan.

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## Conflict of interest

None

## Authors' contributions

All authors contributed to the design of the study and data collection, and participated in the writing of the manuscript and all agree to accept equal responsibility for the accuracy of the contents of this paper.

## Supplementary material available online

Supplementary Table 1-2.

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ORIGINAL ARTICLE

# Validation of different sets of criteria for the diagnosis of Sjögren's syndrome in Japanese patients

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**Abstract**
*Objective* To validate the revised Japanese Ministry of Health criteria for the diagnosis of Sjögren's syndrome (SS) (JPN) (1999), The American-European Consensus Group classification criteria for SS (AECG) (2002), and American College of Rheumatology classification criteria for SS (ACR) (2012).
*Methods* The study subjects were 694 patients with SS or suspected SS who were followed-up in June 2012 at ten hospitals that form part of the Research Team for Autoimmune Diseases, The Research Program for Intractable Disease by the Ministry of Health, Labor and Welfare (MHLW). All patients had been checked for all four criteria of the JPN (pathology, oral, ocular, anti-SS-A/SS-B antibodies). We studied the clinical diagnosis made by the physician in charge and the satisfaction of the above criteria.
*Results* Of the 694 patients, 499 patients did not have other connective tissue diseases (CTDs). SS was diagnosed in 476 patients (primary SS in 302, secondary SS in 174), whereas non-SS was diagnosed in 218 patients (without other CTDs in 197, with other CTDs in 21) by the physician in charge. The sensitivities of JPN, AECG, and ACR in the diagnosis of all forms of SS (both primary and secondary SS) were 79.6, 78.6, and 77.5 %, respectively, with respective specificities of 90.4, 90.4, and 83.5 %. The sensitivities of the same systems in the diagnosis of primary SS were 82.1, 83.1, and 79.1 %, respectively, with specificities of 90.9, 90.9, and 84.8 %, respectively. The sensitivities of the same systems in the diagnosis of secondary SS were 75.3, 70.7, and 74.7 %, respectively, with specificities of 85.7, 85.7, and 71.4 %, respectively.
*Conclusion* The sensitivity of JPN to all forms of SS and secondary SS, the sensitivity of AECG to primary SS, and the specificities of JPN and AECG for all forms of SS, primary SS, and secondary SS were highest in the diagnosis of SS in Japanese patients. These results indicate that

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the JPN criteria for the diagnosis of SS in Japanese patients are superior to ACR and AECG.

**Keywords** Sjögren's syndrome · Criteria

## Introduction

Sjögren's syndrome (SS) is an autoimmune disease that affects exocrine glands, including the salivary and lacrimal glands. It is characterized by lymphocytic infiltration into the exocrine glands, leading to dry mouth and eyes. A number of autoantibodies, such as anti-SS-A and SS-B antibodies, are detected in patients with SS. SS is subcategorized into primary SS, which is not associated with other well-defined connective tissue diseases (CTDs), and secondary SS, which is associated with other well-defined CTDs [1]. Primary SS is further subcategorized into the glandular form and the extraglandular form.

The revised criteria for the diagnosis of SS issued by the Japanese Ministry of Health (JPN) (1999) (Table 1) [2], as well as the American-European Consensus Group classification criteria for SS (AECG) (2002) (Tables 2, 3) [1], are usually used in both daily clinical practice and clinical studies in Japan. Thus, two sets of diagnostic systems are being applied for the same disease. This could result in a heterogeneous pool of SS patients. This heterogeneity of SS patients makes it difficult to analyze the diagnosis, efficacy of treatment, and prognosis of SS patients. A better alternative would be to use a unified set of criteria for the diagnosis of SS in Japan. Recently, The American College of Rheumatology (ACR) published the ACR classification criteria for SS (2012) (Table 4), which were proposed by the Sjögren's International Collaborative Clinical Alliance (SICCA) [3]. The new set of criteria is designed to be used worldwide, not only in advanced countries but also in developing countries. The SICCA established a uniform classification for SS based on a combination of objective tests that have known specificity to SS [3].

**Table 1** The revised Japanese Ministry of Health criteria for the diagnosis of SS (1999)

| |
|---|
| 1. Histopathology |
| Definition: Positive for at least one of (A) or (B) |
| (A) Focus score ≥1 (periductal lymphoid cell infiltration ≥50) in a 4 $mm^2$ minor salivary gland biopsy |
| (B) Focus score ≥1 (periductal lymphoid cell infiltration ≥50) in a 4 $mm^2$ lacrimal gland biopsy |
| 2. Oral examination |
| Definition: Positive for at least one of (A) or (B) |
| (A) Abnormal findings in sialography ≥stage 1 (diffuse punctate shadows of <1 mm) |
| (B) Decreased salivary secretion (flow rate ≤10 ml/10 min according to the chewing gum test or ≤2 g/2 min according to the Saxon test) and decreased salivary function according to salivary gland scintigraphy |
| 3. Ocular examination |
| Definition: Positive for at least one of (A) or (B) |
| (A) Schirmer's test ≤5 mm/5 min and rose bengal test ≥3 according to the van Bijsterveld score |
| (B) Schirmer's test ≤5 mm/5 min and positive fluorescein staining test |
| 4. Serological examination |
| Definition: Positive for at least one of (A) or (B) |
| (A) Anti-Ro/SS-A antibody |
| (B) Anti-La/SS-B antibody |
| Diagnostic criteria: diagnosis of SS can be made when the patient meets at least two of the above four criteria |

Upon comparing these three classification sets, there are some differences among them in their purpose and the items adopted in the set (Table 5). The JPN criteria (1999) are intended as an aid for diagnosis, whereas the AECG criteria (2002) and the ACR criteria (2012) are intended for classification purposes in clinical studies and trials. Although the ACR criteria include only three objective items (Tables 4, 5) and are the simplest among the three sets, the ACR criteria may not identify SS patients with negative findings in labial salivary gland biopsy, because the ACR criteria do not include salivary secretion analysis and imaging studies. On the other hand, the JPN criteria combined oral examinations such as salivary secretion, sialography, and salivary gland scintigraphy with three objective items adopted in the ACR criteria (Table 5). Only the AECG criteria include ocular and oral symptoms, which may cause false positives in patients with non-SS conditions such as aging or visual display terminals (VDT) syndrome (Table 5).

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**Table 2** The American-European Consensus Group classification criteria for SS (2002)

| |
|---|
| I. Ocular symptoms: a positive response to at least one of the following questions |
| 1. Have you had daily, persistent, troublesome dry eyes for more than 3 months? |
| 2. Do you have a recurrent sensation of sand or gravel in the eyes? |
| 3. Do you use tear substitutes more than 3 times a day? |
| II. Oral symptoms: a positive response to at least one of the following questions |
| 1. Have you had a daily feeling of dry mouth for than 3 months? |
| 2. Have you had recurrently or persistently swollen salivary glands as an adult? |
| 3. Do you frequently drink liquids to aid in swallowing dry food? |
| III. Ocular signs—that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests |
| 1. Schirmer's test, performed without anaesthesia ($\leq$5 mm in 5 min) |
| 2. Rose bengal score or other ocular dry eye score ($\geq$4 according to van Bijsterveld's scoring system) |
| IV. Histopathology: in minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score $\geq$1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acuni and contain more than 50 lymphocytes) per 4 $mm^2$ of glandular tissue |
| V. Salivary gland involvement: objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests |
| 1. Unstimulated whole salivary flow ($\leq$1.5 ml in 15 min) |
| 2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts |
| 3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer |
| VI. Autoantibodies: presence in the serum of the following autoantibodies |
| 1. Antibodies to Ro (SS-A) or La (SS-B) antigens, or both |

**Table 3** The American-European Consensus Group classification criteria for SS (2002) rules for classification

| |
|---|
| For primary SS |
| In patients without any potentially associated disease, primary SS may be defined as follows: |
| (A) The presence of any 4 of the 6 items is indicative of primary SS, as long as either item IV (histopathology) or VI (serology) is positive |
| (B) The presence of any 3 of the 4 objective criteria items (that is, items III, IV, V, VI) |
| For secondary SS |
| In patients with a potentially associated disease (for instance, another well-defined connective tissue disease), the presence of item I or item II plus any 2 from among items III, IV, and V may be considered as indicative of secondary SS |
| Exclusion criteria: |
| Past head and neck radiation treatment |
| Hepatitis C infection |
| Acquired immunodeficiency disease (AIDS) |
| Pre-existing lymphoma |
| Sarcoidosis |
| Graft vs. host disease |
| Use of anticholinergic drugs (for a time shorter than 4-fold the half life of the drug) |

The purpose of the present study was to validate the JPN criteria, AECG criteria, and ACR criteria for the diagnosis of SS in Japanese patients. The study identified the differences among these three classification sets.

## Patients and methods

### Study population

The study subjects were 694 patients (51 males and 643 females) with a diagnosis of SS or suspected SS who had been checked for all four criteria of the JPN (pathology, oral, ocular, anti-SS-A/SS-B antibody), and were followed up in June 2012 at ten hospitals across Japan (Kanazawa Medical University Hospital, Nagasaki University Hospital, Hyogo Medical University Hospital, Keio University Hospital, Tokyo Women's Medical University Hospital, Tsurumi University Hospital, Kyushu University Hospital, University of Occupational and Environmental Health Hospital, Kyoto University Hospital, and University of Tsukuba Hospital) that form part of the Research Team for Autoimmune Diseases, The Research Program for Intractable Disease of the Ministry of Health, Labor and Welfare (MHLW).

### Data collection and analysis

We collected clinical data from the above ten hospitals using a questionnaire. We retrospectively examined the clinical diagnosis made by the physician in charge, as well as the satisfaction of the JPN, AECG, and ACR criteria. Because lissamine green ocular staining had not been adopted in Japan at the time of clinical examination, we regarded patients who had a positive rose bengal test or fluorescein staining test as having satisfied the ocular staining score in the ACR classification system.

We regarded the clinical diagnosis made by the physician in charge as the gold standard for the diagnosis of SS in this study. We compared the sensitivities and specificities of the JPN, AECG, and ACR diagnostic systems in the diagnosis of SS (both primary and secondary SS), primary

**Table 4** The American College of Rheumatology classification criteria for SS (2012)

| |
|---|
| The classification of SS, which applies to individuals with signs/symptoms that may be suggestive of SS, will be met in patients who have at least 2 of the following 3 objective features: |
| 1. Positive serum anti-SS-A/Ro and/or anti-SS-B/La or (positive rheumatoid factor and ANA titer $\geq$1:320) |
| 2. Labial salivary gland biopsy exhibiting focal lymphocytic sialadenitis with a focus score $\geq$1 focus/4 $mm^2$ |
| 3. Keratoconjunctivitis sicca with ocular staining score $\geq$3 (assuming that individual is not currently using daily eye drops for glaucoma and has not had corneal surgery or cosmetic eyelid surgery in the last 5 years) |
| Prior diagnosis of any of the following conditions would exclude participation in SS studies or therapeutic trials because of overlapping clinical features or interference with criteria tests: |
| History of head and neck radiation treatment |
| Hepatitis C infection |
| Acquired immunodeficiency syndrome |
| Sarcoidosis |
| Amyloidosis |
| Graft vs. host disease |
| IgG4-related disease |

SS, and secondary SS. Agreement between the three was assessed via the kappa coefficient.

## Results

### Diagnosis of SS (primary and secondary SS) and non-SS

Of the 694 patients, 499 patients did not have other well-defined CTDs, whereas 195 patients did. SS was diagnosed in 476 patients (302 primary SS, 174 secondary SS), whereas non-SS was diagnosed in 218 patients (197 without other CTDs, 21 with other CTDs) by the physician in charge (Table 6).

### Sensitivities and specificities of the three diagnostic systems for SS

The sensitivities of JPN, AECG, and ACR in the diagnosis of all SS (302 primary SS and 174 secondary SS) were 79.6, 78.6, and 77.5 %, respectively, whereas the respective specificities in the diagnosis of all SS were 90.4, 90.4, and 83.5 %. The sensitivities of JPN, AECG, and ACR in the diagnosis of 302 primary SS were 82.1, 83.1, and 79.1 %, respectively, with specificities of 90.9, 90.9, and 84.8 %, respectively. The sensitivities of JPN, AECG, and ACR in the diagnosis of 174 secondary SS were 75.3, 70.7, and 74.7 %, respectively, with specificities of 85.7, 85.7, and 71.4 % (Table 7).

**Table 5** Comparison of the items adopted in the JPN and AECG and ACR criteria

| | JPN | AECG | ACR |
|---|---|---|---|
| Ocular symptoms | × | ○ | × |
| Oral symptoms | × | ○ | × |
| Ocular signs | | | |
| Schirmer's test | ○ | ○ | × |
| Ocular staining | ○ | ○ | ○ |
| Labial salivary gland biopsy | ○ | ○ | ○ |
| Salivary gland involvements | | | |
| Salivary secretion | ○ | ○ | × |
| Sialography | ○ | ○ | × |
| Scintigraphy | ○ | ○ | × |
| Autoantibodies | | | |
| SS-A | ○ | ○ | ○ |
| SS-B | ○ | ○ | ○ |
| ANA | × | × | ○ |
| RF | × | × | ○ |

*SS-A* anti-SS-A antibody, *SS-B* anti-SS-B antibody, *ANA* anti-nuclear antibody, *RF* rheumatoid factor, ○ adopted, × not adopted, *JPN* the revised Japanese Ministry of Health criteria for the diagnosis of Sjögren's syndrome (1999), *AECG* The American-European Consensus Group classification criteria for Sjögren's syndrome (2002), *ACR* American College of Rheumatology classification criteria for Sjögren's syndrome (2012)

**Table 6** Diagnosis of SS and non-SS

| | Associated with other CTDs | | Total |
|---|---|---|---|
| | No | Yes | |
| Clinical diagnosis | | | |
| SS | 302 (primary SS) | 174 (secondary SS) | 476 |
| Non-SS | 197 | 21 | 218 |
| Total | 499 | 195 | 694 |

*Clinical diagnosis* diagnosis of SS by the physician in charge
*CTDs* connective tissue diseases

### Comparisons of the satisfaction of the three diagnostic systems

Figure 1 displays Venn diagrams showing comparisons of the satisfaction of the three diagnostic systems. Among all SS patients ($n = 476$), more patients satisfied only the AECG criteria ($n = 42$) rather than only the JPN criteria ($n = 8$) or the ACR criteria ($n = 6$). The same tendency was also observed in patients with primary SS only and in those with secondary SS only. The diagrams indicate that the JPN and ACR diagnostic systems are similar, whereas the AECG diagnostic system is different from the other two. Table 8 shows the agreement among the three

**Table 7** Sensitivities and specificities of the three tested systems for diagnosing SS

| | Entire group | | Without other CTDs | | With other CTDs | |
|---|---|---|---|---|---|---|
| | Sensitivity | Specificity | Sensitivity | Specificity | Sensitivity | Specificity |
| JPN | 79.6 | 90.4 | 82.1 | 90.9 | 75.3 | 85.7 |
| AECG | 78.6 | 90.4 | 83.1 | 90.9 | 70.7 | 85.7 |
| ACR | 77.5 | 83.5 | 79.1 | 84.8 | 74.7 | 71.4 |

The "entire group" comprised 694 patients, including 476 with SS (302 patients with primary SS and 174 with secondary SS) and 218 patients with non-SS. The "without other CTDs" group of 499 patients included 302 patients with primary SS and 197 with non-SS. The "with other CTDs" group of 195 patients included 174 patients with secondary SS and 21 with non-SS

*JPN* Japanese Ministry of Health criteria for the diagnosis of Sjögren's syndrome (1999), *AECG* The American-European Consensus Group classification criteria for Sjögren's syndrome (2002), *ACR* The American College of Rheumatology classification criteria for Sjögren's syndrome (2012)

diagnostic systems, as assessed using the kappa coefficient. The data indicate a high level of agreement between the JPN and ACR diagnostic systems (kappa coefficient 0.74), but a low level of agreement between AECG and the other two (kappa coefficient 0.10–0.46) in the diagnosis of all SS, primary SS, and secondary SS.

## Discussion

While it is difficult to select the best gold standard system for the diagnosis of CTDs such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and SS, this issue is clinically relevant and important. In SLE, the ACR revised criteria for the classification of SLE (1997) [4] has been adopted for diagnosis in daily clinical practice and for classification purposes in clinical studies. Recently, the Systemic Lupus International Collaborating Clinics (SLICC) has proposed new classification criteria for SLE [5], which has generated interesting discussion about these two criteria among expert rheumatologists. On the other hand, for RA, the 2010 RA classification criteria: an ACR/European League Against Rheumatism (EULAR) collaborative initiative [6] was published recently and is currently used not only in clinical studies for the classification of RA but also in daily clinical practice for the diagnosis of RA. Therefore, these available diagnostic systems for SLE and RA could be regarded as the gold standard for both clinical studies and daily clinical practice. The AECG criteria have been adopted in Western countries for the diagnosis of SS. In Japan, however, both the AECG and JPN criteria are currently being used simultaneously for the classification and diagnosis of SS. On the other hand, the new ACR criteria have been proposed as a uniform classification for SS. At present, there is no gold standard system for the diagnosis of SS in both clinical studies and daily clinical practice, except for expert judgment. This state could create a heterogeneous pool of SS patients, which makes it difficult to analyze the diagnosis, efficacy of treatment, and prognosis of SS patients. Establishing a single set of criteria for SS and selecting a gold standard system for the diagnosis of SS is an important task in Japan.

The present study demonstrated that the sensitivity of the JPN system for all SS and secondary SS, the sensitivity of the AECG system for primary SS, and the specificities of the JPN and AECG systems for all SS, primary SS, and secondary SS were highest among the three systems for diagnosing SS in Japanese patients (relative to clinical judgment as the gold standard). The results also showed high agreement between the JPN and ACR systems, but low agreement between AECG and the other two diagnostic systems for all SS, primary SS, and secondary SS. These results indicate that the JPN and ACR criteria covered similar patient populations, although the sensitivity and specificity were higher for the JPN system than the ACR system. Among the 302 patients with primary SS, 14 did not satisfy the ACR criteria for the diagnosis of SS, although they did meet the criteria of both JPN and AECG. Further analysis of these 14 SS patients also showed that 50 % of these patients had negative pathological findings, 70 % had negative ocular staining, and 50 % were negative for autoantibodies (data not shown). These SS patients could be misdiagnosed by the ACR criteria, resulting in the lower sensitivity of the ACR diagnostic system. On the other hand, among 197 non-SS patients without other CTDs, ten patients satisfied the ACR criteria but not the JPN nor the AECG criteria (data not shown). Further analysis of these ten patients indicated that 80 % were positive for lissamine green ocular staining (Schirmer's test, rose bengal staining, and fluorescein staining were not performed), and 60 % were positive for anti-SS-A antibody (data not shown). Although these patients might be misdiagnosed as primary SS by the ACR criteria, this could not be confirmed because these patients could be positive for other ocular tests adopted by the JPN and AECG diagnostic systems.

The specificities of the criteria for all SS, primary SS, and secondary SS patients used in the JPN and AECG

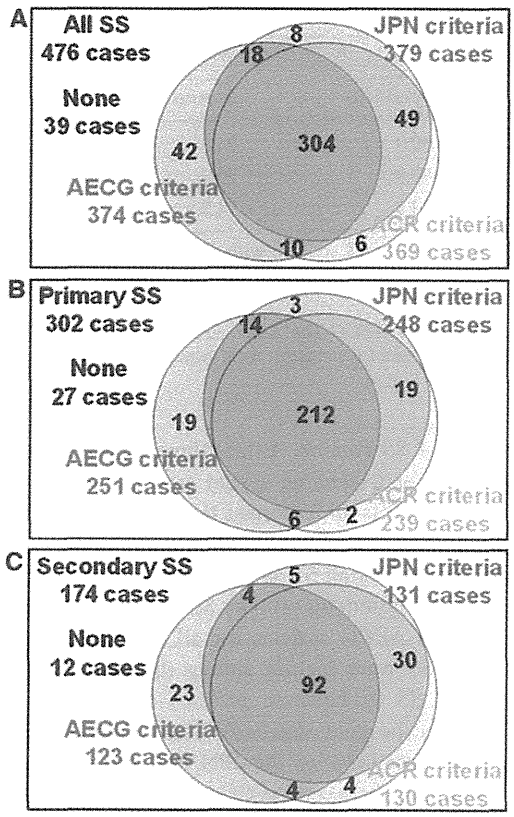


**Fig. 1** Venn diagrams showing a comparison of the satisfaction of the three tested systems. **a** Comparison of the satisfaction of the three tested systems, performed using data from all 476 SS patients (302 primary SS and 174 secondary SS). **b** Comparison of the satisfaction of the three tested systems using data on 302 patients with primary SS. **c** Comparison of the satisfaction of the three tested systems using data on 174 patients with secondary SS. *Numbers* show the numbers of patients who satisfied each set of criteria, *None* indicates the number of patients who did not satisfy the criteria of any of the three systems. *JPN criteria* the revised Japanese Ministry of Health criteria for the diagnosis of SS (1999), *AECG criteria* The American-European Consensus Group classification criteria for SS (2002), *ACR criteria* American College of Rheumatology classification criteria for SS (2012)

systems were the same in this study. The reason for the same specificities of the JPN and AECG criteria may be the identical number of non-SS patients (21 patients, including 18 patients without CTDs and 3 patients with CTDs) who satisfied JPN and AECG. However, the JPN and AECG profiles for 20 out of these 21 non-SS patients were completely different, highlighting the low agreement between JPN and AECG, as shown in Table 8.

The sensitivity of AECG for primary SS was highest among the three systems, whereas that of JPN for all SS and secondary SS was highest. Among the 302 primary SS patients, 19 patients only satisfied the AECG criteria. These 19 primary SS patients had high frequencies of dry eye (84.2 %) and dry mouth (100.0 %) but low frequencies of anti-SS-A antibody (10.5 %) and anti-SS-B antibody (0 %). These seronegative primary SS patients with symptoms of dryness could only be diagnosed by the AECG criteria, because only the AECG criteria include symptoms of dryness. This may be the sensitivity of AECG for primary SS was highest among the three systems.

The above findings suggest that JPN provided the best set of criteria necessary for the diagnosis of Japanese patients with SS. Admittedly, however, the results of the present study do not allow us to confirm the superiority of JPN due to the inherent limitations of the study. First, we used the clinical judgment of the physician in charge as the gold standard. In Japan, because the JPN criteria are the criteria used most commonly in daily clinical practice, the clinical judgment could depend on the satisfaction of the JPN criteria. It is better to rely on expert committee consensus based on clinical case scenarios as the gold standard for diagnosis in order to avoid this bias. Second, patients who had been checked for all four criteria of the JPN diagnostic system (pathology, oral, ocular, anti-SS-A/SS-B antibodies) were included in this study, but the methods used for ocular staining varied among the participating institutions. Third, the results of the study could include selection bias. For these reasons, we need a more

**Table 8** Agreement among the three tested systems, as assessed using the kappa coefficient

| All SS ($n$ = 476) | All SS ($n$ = 476) (primary SS, $n$ = 302, secondary SS, $n$ = 174) | Primary SS ($n$ = 302) | Secondary SS ($n$ = 174) |
|---|---|---|---|
| JPN vs. AECG | 0.31 | 0.46 | 0.10 |
| JPN vs. ACR | 0.74 | 0.74 | 0.74 |
| AECG vs. ACR | 0.30 | 0.42 | 0.12 |

The "entire group" comprised 694 patients, including 476 with SS (302 patients with primary SS and 174 with secondary SS) and 218 patients with non-SS. The "without other CTDs" group of 499 patients included 302 patients with primary SS and 197 with non-SS. The "with other CTDs" group of 195 patients included 174 patients with secondary SS and 21 with non-SS.

*JPN* Japanese Ministry of Health criteria for the diagnosis of Sjögren's syndrome (1999), *AECG* The American-European Consensus Group classification criteria for Sjögren's syndrome (2002), *ACR* The American College of Rheumatology classification criteria for Sjögren's syndrome (2012)

sophisticated validation study using randomly selected clinical case scenarios from various institutions and expert committee consensus diagnosis as the golden standard to test the three diagnostic systems for SS, to unify the criteria used for the diagnosis of SS, and ultimately to select the gold standard set of criteria for the diagnosis of SS in Japan.

Currently, the JPN diagnostic system is only used in Japan, because ACR and EULAR have never validated the JPN system. Therefore, we strongly hope that an ACR/EULAR collaborative initiative will validate JPN as well as the AECG and ACR systems.

In conclusion, although this study has a few limitations, the results obtained from it indicate the superiority of the JPN criteria, as it has higher sensitivity and specificity values for the diagnosis of SS in Japanese patients with SS than those of ACR and AECG.

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**Conflict of interest** None.

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# A Genome-Wide Association Study Identified *AFF1* as a Susceptibility Locus for Systemic Lupus Eyrthematosus in Japanese

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## Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease that causes multiple organ damage. Although recent genome-wide association studies (GWAS) have contributed to discovery of SLE susceptibility genes, few studies has been performed in Asian populations. Here, we report a GWAS for SLE examining 891 SLE cases and 3,384 controls and multi-stage replication studies examining 1,387 SLE cases and 28,564 controls in Japanese subjects. Considering that expression quantitative trait loci (eQTLs) have been implicated in genetic risks for autoimmune diseases, we integrated an eQTL study into the results of the GWAS. We observed enrichments of cis-eQTL positive loci among the known SLE susceptibility loci (30.8%) compared to the genome-wide SNPs (6.9%). In addition, we identified a novel association of a variant in the AF4/FMR2 family, member 1 (*AFF1*) gene at 4q21 with SLE susceptibility (rs340630; $P = 8.3 \times 10^{-9}$, odds ratio = 1.21). The risk A allele of rs340630 demonstrated a cis-eQTL effect on the *AFF1* transcript with enhanced expression levels ($P < 0.05$). As *AFF1* transcripts were prominently expressed in $CD4^+$ and $CD19^+$ peripheral blood lymphocytes, up-regulation of *AFF1* may cause the abnormality in these lymphocytes, leading to disease onset.

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## Author Summary

Although recent genome-wide association study (GWAS) approaches have successfully contributed to disease gene discovery, many susceptibility loci are known to be still uncaptured due to strict significance threshold for multiple hypothesis testing. Therefore, prioritization of GWAS results by incorporating additional information is recommended. Systemic lupus erythematosus (SLE) is an autoimmune disease that causes multiple organ damage. Considering that abnormalities in B cell activity play essential roles in SLE, prioritization based on an expression quantitative trait loci (eQTLs) study for B cells would be a promising approach. In this study, we report a GWAS and multi-stage replication studies for SLE examining 2,278 SLE cases and 31,948 controls in Japanese subjects. We integrated eQTL study into the results of the GWAS and identified *AFF1* as a novel SLE susceptibility loci. We also confirmed cis-regulatory effect of the locus on the *AFF1* transcript. Our study would be one of the initial successes for detecting novel genetic locus using the eQTL study, and it should contribute to our understanding of the genetic loci being uncaptured by standard GWAS approaches.


## Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production, complement activation, and multi-organ damage [1]. Familial aggregation demonstrates that both genetic and environmental factors play a role in pathogenesis of SLE [2]. Genetic studies using candidate gene-approaches, and recently, genome-wide association studies (GWAS), have uncovered more than 25 SLE susceptibility genes, including *HLA-DRB1*, *IRF5*, *STAT4*, *ITGAM*, *BLK*, *TNFAIP3*, and others [3–18]. However, most of these studies were conducted in European populations [3–13,15,17], and few studies have been conducted in Asian populations [14,16,18]. Since the epidemiology of SLE has demonstrated that the prevalence of disease substantially differs among populations, genetic backgrounds of SLE should be also heterogeneous across populations [19,20]. Therefore, additional studies in Asians might provide novel insights. It is of note that GWAS for SLE in Chinese populations identified novel loci that had not been detected in Europeans, such as *ETS1*, *IKZF1*, and *WDFY4* [14,16].

Another issue raised by the previous GWASs for complex diseases is that many susceptibility loci still remained uncaptured, owing to its strict significance threshold for multiple hypothesis testing [21]. In SLE, for example, the 26 risk loci identified by the previous GWAS explained only an estimated 8% of the total genetic susceptibility to the disease [15]. Therefore, it is still important to examine the sub-loci of GWAS, in order to reveal the entire picture of genetic etiology. To effectively explore these uncaptured loci, prioritization of GWAS results by incorporating additional information implicated in the disease pathophysiology is recommended [22,23]. Considering that abnormalities in B cell activity play essential roles in SLE [1] and that expression quantitative trait loci (eQTL) have been implicated to comprise approximately a half of genetic risks for autoimmune diseases [24], prioritization based on an eQTL study for B cells would be a promising approach for SLE [25]. Moreover, an eQTL itself assures the presence of functional variant(s) that regulate gene expression. Thus, eQTL increases the prior probability of the presence of disease-causal variant(s) in the locus more effectively and unbiasedly, compared to other knowledge-based prioritizations such as gene pathway analysis [24].

Here, we report a GWAS and multi-stage replication studies for SLE examining 2,278 SLE cases and 31,948 controls in Japanese subjects. We integrated eQTL study into the results of the GWAS, which effectively enabled to detect a novel SLE susceptibility locus.

## Results

### GWAS for SLE

In the GWAS, 891 SLE cases and 3,384 controls in Japanese subjects were genotyped over 550,000 single nucleotide polymorphism (SNP) markers (Table S1, S2 and Figure 1). We applied stringent quality control (QC) criteria and evaluated associations of 430,797 autosomal SNPs, as previously described [26]. No substantial population stratification was demonstrated through principal component analysis (Figure S1) or a Quantile–Quantile plot of *P*-values (inflation factor, $\lambda_{GC,} = 1.088$, Figure S2), suggesting homogenous ancestries of our study population [27].

We identified significant associations in six chromosomal loci that satisfied the genome-wide significance threshold of $P<5.0\times10^{-8}$ (Table 1 and Figure 2A). These loci have been reported to be associated with SLE susceptibility (*STAT4*, *TNFAIP3*, *HIP1*, *BLK*, *ETS1*, and the HLA region) [3–18]. We also observed significant replications in 17 of the previously reported SLE susceptibility loci [3–18] ($\alpha = 0.01$; Table 2). Of these, significant replications were enriched in the loci identified through the studies in Asian populations (80%; 8 of the 10 loci), including *RASGRP3*, *IKZF1*, *HIP1*, *WDFY4*, intergenic region at 11q23, *ETS1*, *SLC15A4*, *ELF1*, and *HIC2-UBE2L3* [14,16,18], compared to those in European populations (56.3%; 9 of the 16 loci) [3–13,15,17].

### Incorporation of eQTL study into GWAS results

For the selection of SNPs incorporated in the replication studies of the potential association signals, we evaluated cis-eQTL effects of the SNPs using publically available gene expression data [28], and prioritized the results of the GWAS. After applying QC criteria, we evaluated the expression levels of 19,047 probes assayed in lymphoblastoid B cell lines from Phase II HapMap East-Asian individuals [29] using Illumina's human whole-genome expression array (WG-6 version 1) [28]. For each of the SNPs included in our GWAS, probes located within ±300 kbp regions were focused on as cis-eQTLs (average 4.93 probes per SNP). We denoted the SNPs which exhibited significant associations with expression levels of any of the corresponding cis-eQTLs as eQTL positive (false discovery rate (FDR) *Q*-values<0.2). We observed enrichments of eQTL positive loci among the SLE susceptibility loci (30.8%; 8 of the 26 evaluated loci) including a well-known eQTL gene of *BLK* [11,25] (Table 2), compared to the genome-wide SNPs (6.9%) and compared even to the SNPs in the vicinity of expressed loci (among the SNPs located within ±10 kbp of probes used for the expression analysis, 13.1% were eQTL positive; Table S3).

By prioritizing the results of the GWAS using the eQTL study, we selected 57 SNPs from 1,207 SNPs that satisfied $P<1.0\times10^{-3}$ in the GWAS. We subsequently referred the associations of the selected SNPs using the results of the concurrent genome-wide scan for SLE in an independent Japanese population (Tahira T et al. Presented at the 59th Annual Meeting of the American Society of Human Genetics, October 21, 2009). In the scan, 447 SLE cases and 680 controls of Japanese origin were evaluated using a pooled DNA approach [30]. We selected SNPs if any association signals were observed in the neighboring SNPs of the

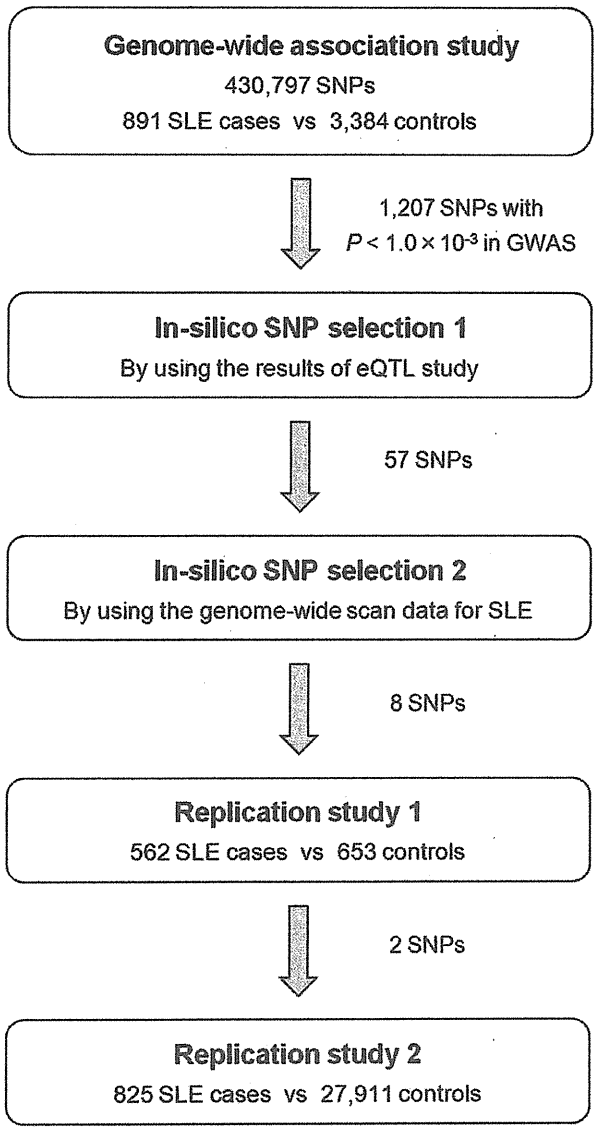


**Figure 1. Design of the GWAS and multi-stage replication studies for SLE in Japanese subjects.** A total of 2,278 SLE cases and 31,948 controls were enrolled. The clinical characteristics of the subjects are summarized in Table S1 and S2. Details of the genome-wide scan data for SLE referenced in the *in silico* SNP selection 2 are described elsewhere (Tahira T et al. Presented at the 59th Annual Meeting of the American Society of Human Genetics, October 21, 2009).
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pooled analysis. As a result, 8 SNPs remained for further investigation (Table S4).

### Replication studies and identification of *AFF1*

Then, we performed two-stage replication studies using independent SLE cohorts for Japanese subjects (cohort 1 with 562 SLE cases and 653 controls, and cohort 2 with 825 SLE cases and 27,911 controls). First, we evaluated the selected 8 SNPs in the replication study 1. In the replication study 2, 2 SNPs that satisfied $P<1.0\times10^{-6}$ in the combined study of GWAS and replication study 1 were further evaluated (Figure 1). Among the evaluated SNPs, we observed significant replications in the SNP located in the genomic region of the AF4/FMR2 family, member 1 gene (*AFF1*) at 4q21 (rs340630; $P=4.6\times10^{-5}$ and $P=0.0094$ in the two individual cohorts, respectively; Table 3, Table S5, and Figure 2B). The combined study for the GWAS ($P=1.5\times10^{-4}$) and the replication studies demonstrated significant associations of rs340630 that satisfied the genome-wide significance threshold ($P=8.3\times10^{-9}$, OR = 1.21, 95% CI 1.14–2.30).

### Cis-eQTL effect of rs340630 on *AFF1* transcripts

Since the landmark SNP in the *AFF1* locus, rs340630, was prioritized through the eQTL study as an eQTL positive SNP (Table 3), we further validated its cis-eQTL effect using Epstein-Barr virus (EBV)-transfected B cell lines established from Japanese individuals (Pharma SNP Consortium (PSC) cells, $n=62$). The correlation between rs340630 genotypes and the expression levels of *AFF1* was significant in the PSC cells stimulated with phorbol myristate acetate (PMA) ($R^2=0.074$, $P=0.033$; Figure 3A). The expression levels increased with the number of SLE-risk (A) alleles. To further confirm this cis-regulatory effect, we performed allele-specific transcript quantification (ASTQ) of *AFF1*. The transcript levels of each allele were quantified by qPCR using an allele specific probe for a SNP in the 5′-untranslated region (rs340638), which was in absolute LD with rs340630 ($r^2=1.0$, $D'=1.0$). We examined PSC-cells ($n=17$) that were heterozygous for both rs340630 and rs340638. The mean ratio of each transcript (A over G allele; the A allele comprises a haplotype with the risk (A) allele of rs340630) were significantly increased to 1.07 compared to the ratio of the amount of DNA (1.00, $P=0.012$) (Figure 3B). These results suggest that rs340630, or SNP(s) in LD with it, are a regulatory variant predisposing SLE susceptibility through increased expression levels of *AFF1*.

### Expression of *AFF1* in $CD4^+$ and $CD19^+$ peripheral blood lymphocytes

*AFF1* is known to be involved in cytogenetic translocations of acute lymphoblastic leukemia (ALL) [31]. Its fusion protein with the mixed-lineage leukemia gene (*MLL*) is implicated in the regulation of transcription and the cell cycle of lymphocytes [31]. To investigate the expression pattern of *AFF1* in normal tissues, we evaluated the transcript levels of *AFF1* in a panel of various tissues. We observed prominent expression of *AFF1* in $CD4^+$ and $CD19^+$ peripheral blood lymphocytes, implying an important role for *AFF1* in helper-T-cells and B-cells (Figure 3C).

## Discussion

Through a GWAS and multi-staged replication studies consisting of 2,278 SLE cases and 31,948 controls in Japanese subjects, our study identified that the *AFF1* locus was significantly associated with SLE susceptibility.

As well as the identification of the novel SLE susceptibility locus, we observed significant replications of associations in the previously reported susceptibility loci. The replications were especially enriched in the loci identified through the studies in Asian populations, compared to those in European populations. Considering the ethnical heterogeneities in the epidemiology of SLE [19,20], these observations suggest the similarities in the genetic backgrounds of SLE shared within Asian populations, and also the existence of the both common and divergent genetic backgrounds encompassed between European and Asian populations.

**Table 1.** Results of a genome-wide association study for Japanese patients with SLE.

| rsID[a] | Chr | Position (bp) | Cytoband | Gene | Allele[b] | No. subjects | | Allele 1 freq. | | OR (95%CI) | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/2 | Case | Control | Case | Control | | |
| rs10168266 | 2 | 191,644,049 | 2q32 | *STAT4* | T/C | 891 | 3,384 | 0.37 | 0.27 | 1.59 (1.42–1.78) | $2.7\times10^{-16}$ |
| rs9501626 | 6 | 32,508,322 | 6p21 | HLA region | A/C | 891 | 3,381 | 0.20 | 0.12 | 1.86 (1.62–2.13) | $1.0\times10^{-18}$ |
| rs2230926 | 6 | 138,237,759 | 6q23 | *TNFAIP3* | G/T | 891 | 3,377 | 0.11 | 0.069 | 1.75 (1.47–2.08) | $1.9\times10^{-10}$ |
| rs6964720 | 7 | 75,018,280 | 7q11 | *HIP1* | G/A | 891 | 3,384 | 0.25 | 0.19 | 1.43 (1.27–1.63) | $1.3\times10^{-8}$ |
| rs2254546 | 8 | 11,381,089 | 8p23 | *BLK* | G/A | 891 | 3,384 | 0.78 | 0.72 | 1.42 (1.61–1.25) | $4.1\times10^{-8}$ |
| rs6590330 | 11 | 127,816,269 | 11q24 | *ETS1* | A/G | 891 | 3,368 | 0.48 | 0.39 | 1.44 (1.30–1.60) | $1.3\times10^{-11}$ |

[a]SNPs that satisfied the threshold of $P<5.0\times10^{-8}$ were indicated.
[b]Based on forward strand of NCBI Build 36.3.
SLE, systemic lupus erythematosus; OR, odds ratio.
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To effectively detect the novel SLE susceptibility locus, we integrated cis-eQTL effects of the SNPs and prioritized the results of the GWAS. In addition to identifying a novel locus for SLE-susceptibility, our study demonstrated approximately 30% of confirmed SLE-susceptibility loci were comprised of cis-eQTLs. We also confirmed cis-regulatory effect of the landmark SNP in the *AFF1* locus, rs340630, on *AFF1* transcripts, which had been prioritized through the eQTL study. These results would suggest that accumulation of quantitative changes in gene expression would accelerate the disease onset of SLE. It would also demonstrate the validity of applying eQTL study in the search of the susceptible genes for SLE or other autoimmune diseases, as previously suggested in the study for celiac disease [24]. To our knowledge, this is one of the initial studies to successfully discover a new locus by prioritizing GWAS results using eQTLs, and should contribute to the approaches assessing genetic loci still being uncaptured by recent large-scaled GWASs due to stringent significance threshold for multiple hypothesis testing [21].

We observed prominent expression levels of *AFF1* in $CD4^+$ and $CD19^+$ peripheral blood lymphocytes, which would imply an important role for *AFF1* in helper-T-cells and B-cells. In fact, *AFF1* is essential for normal lymphocyte development, as demonstrated in mice deficient for *AFF1*; severe reduction were observed in the thymic double positive CD4/CD8 population and the bone marrow pre-B and mature B-cell numbers [32]. The risk A allele of rs340630 demonstrated a cis-eQTL effect on the *AFF1* transcript with enhanced expression levels. As the *AFF1* locus was also demonstrated as an eQTL in primary liver cells [33], the cis-regulatory effect may hold in primary cells as well as lymphoblastoid cells used in the present study. However, because the mechanism of transcriptional regulation is substantially different among cell types [34], cell-type specific analyses including those for primary T-cells and B-cells are needed for understanding the precise role of *AFF1* variant in primary lymphocytes. Although further functional investigation is necessary, our observation suggested that *AFF1* is involved in the etiology of SLE through the regulation of development and activity of lymphocytes. It is of note that *AFF3*, which also belongs to the AF4/FMR2 family, is associated with susceptibility to autoimmune diseases [35].

One of our study's limitations is the selection of SNPs for the replication study using the results of the pooled DNA approach [30], which used a different genotyping platform from that of the present GWAS. Moreover, the association signals based on Silhouette scores in pooled analysis would be less reliable compared to those based on individual genotyping. Since direct comparisons of the association signals of the same single SNPs between the studies would be difficult due to these issues, we adopted the complementary approach that referred the association signals of the multiple SNPs in the pooled analysis for each of the single SNPs in the GWAS, taking account of LD and physical distances between the SNPs. However, there would exist a possibility that the variant(s) truly associated with SLE was left not to be examined in the replication study. It should be noted that only 1 SNP among the 8 selected SNPs yielded the significant association with SLE, although further enrichments of the significant associations might be anticipated. To elucidate effectiveness and limitation of our approach, further assessments of the studies on the remaining loci would be desirable. It should also be noted that the control-case ratio of the subjects were relatively high in the replication study 2 (= 33.8), and this disproportionate ratio could have induced potential bias on the results of the association analysis of the SNPs. However, considering the homogeneous ancestries of the Japanese population [27] and that principal component analysis did not demonstrate significant population stratification in the control subjects of the replication study 2 (data not shown), the bias owing to population stratification might not be substantial.

In summary, through a GWAS and multi-staged replication studies in a Japanese population integrating eQTL study, our study identified *AFF1* as a novel susceptibility locus for SLE.

## Materials and Methods

### Subjects

We enrolled 2,278 systemic lupus erythematosus (SLE) cases and 31,948 controls. SLE cases enrolled in the genome-wide association study (GWAS) ($n = 891$) or part of the 2nd replication study ($n = 83$) were collected from 12 medical institutes in Japan under the support of the autoimmune disease study group of Research in Intractable Diseases, Japanese Ministry of Health, Labor and Welfare: Hokkaido University Graduate School of Medicine, Tohoku University Graduate School of Medicine, the University of Tokyo, Keio University School of Medicine, Juntendo University School of Medicine, University of Occupational and Environmental Health, University of Tsukuba, Tokyo Medical and Dental University, National Center for Global Health and Medicine, Nagasaki University, Wakayama Medical University, and Jichi Medical University. SLE cases ($n = 562$) and controls ($n = 653$) enrolled in the 1st replication study were collected from Kyushu University. Some of the SLE cases ($n = 742$) and controls ($n = 27,911$) enrolled in the 2nd replication study were collected from Kyoto University, Tokyo Women's

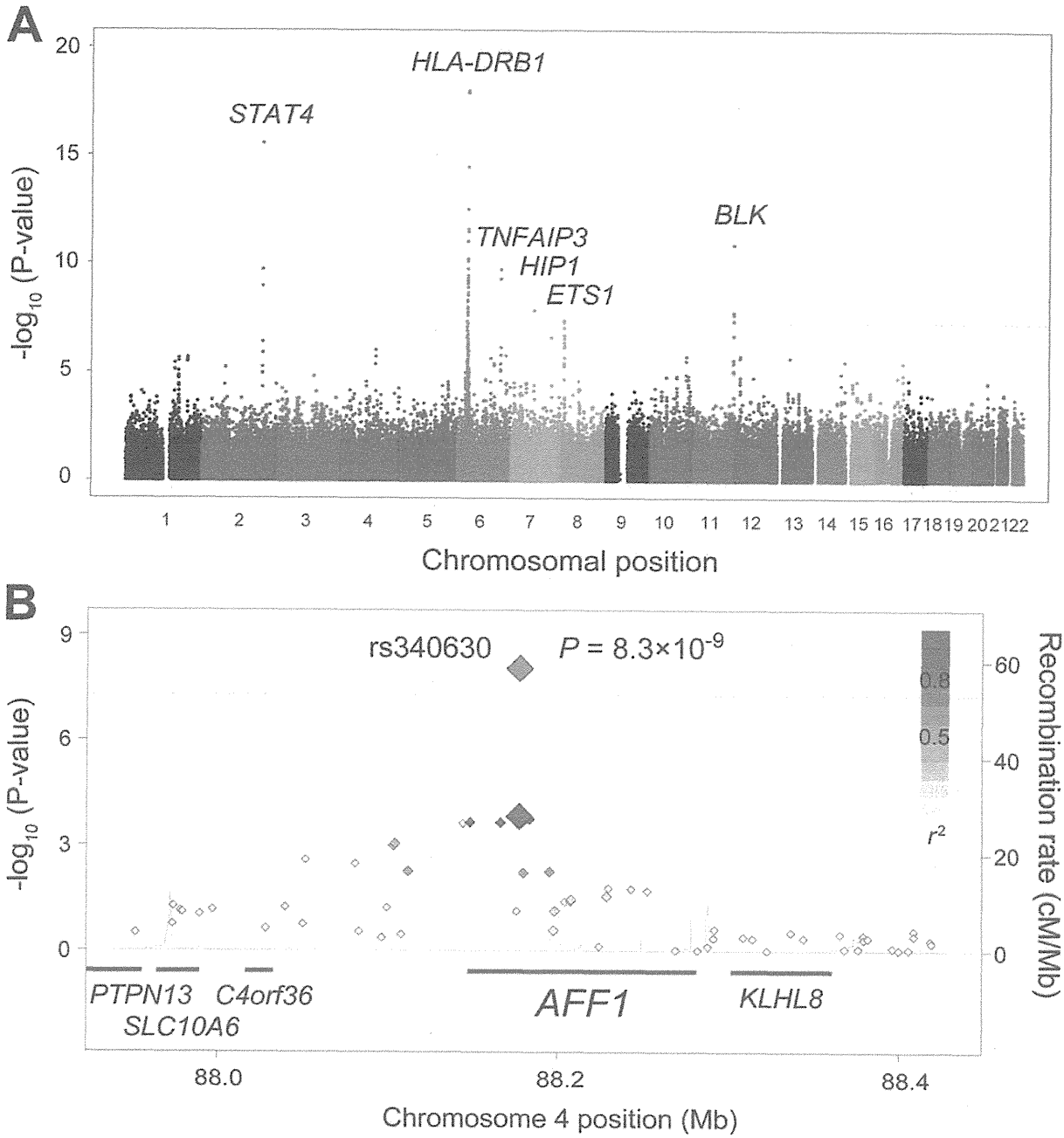


**Figure 2. Associations of the *AFF1* locus with SLE.** (A) A chromosomal plot of *P*-values in GWAS for SLE. (B) A regional plot in the *AFF1* locus. Diamond-shaped data points represent $-\log_{10}$ (*P*-values) of the SNPs. Large-sized points indicate the *P*-values of the landmark SNP, rs340630 (green for the combined study and red for the GWAS). Density of red color represents $r^2$ values with rs340630. Blue line represents recombination rates. Lower part indicates RefSeq genes. Gray dashed horizontal lines represent the threshold of $P = 5.0 \times 10^{-8}$. The plots were drawn using SNAP, version 2.1 [47].
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Medical University, the University of Tokyo, and the BioBank Japan Project [36]. All subjects were of Japanese origin and provided written informed consent. SLE cases met the revised American College of Rheumatology (ACR) criteria for SLE [37]. Control subjects were confirmed to be free of autoimmune disease. Some of the SLE cases were included in our previous studies [38–40]. Details of the subjects are summarized in Table S1 and S2. This research project was approved by the ethical committees of the University of Tokyo, RIKEN, and affiliated medical institutes.

**Table 2.** Associations among previously reported SLE-related loci.

| rsID | Chr | Position (bp) | Cytoband | Gene | Allele[a] | Allele 1 freq. | | OR (95%CI) | P | eQTL[b] | Identified by the studies in[c] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/2 | Case | Control | | | | Caucasians | Asians |
| rs2205960 | 1 | 171,458,098 | 1q25 | *TNFSF4* | T/G | 0.23 | 0.18 | 1.35 (1.19–1.54) | $3.0\times10^{-6}$ | | + | |
| rs3024505 | 1 | 205,006,527 | 1q32 | *IL10* | A/G | 0.019 | 0.014 | 1.34 (0.90–2.00) | 0.15 | | + | |
| rs13385731 | 2 | 33,555,394 | 2p22 | *RASGRP3* | C/T | 0.90 | 0.87 | 1.37 (1.15–1.64) | $6.0\times10^{-4}$ | + | | + |
| rs10168266 | 2 | 191,644,049 | 2q32 | *STAT4* | T/C | 0.37 | 0.27 | 1.59 (1.42–1.78) | $2.7\times10^{-16}$ | | + | |
| rs6445975 | 3 | 58,345,217 | 3p14 | *PXK* | G/T | 0.25 | 0.23 | 1.09 (0.96–1.23) | 0.18 | + | + | |
| rs10516487 | 4 | 102,970,099 | 4q24 | *BANK1* | G/A | 0.91 | 0.89 | 1.28 (1.07–1.53) | 0.0070 | | + | |
| rs10036748 | 5 | 150,438,339 | 5q33 | *TNIP1* | T/C | 0.75 | 0.72 | 1.16 (1.03–1.31) | 0.014 | | | + |
| rs9501626 | 6 | 32,508,322 | 6p21 | *HLA-DRB1* | A/C | 0.20 | 0.12 | 1.86 (1.62–2.13) | $1.0\times10^{-18}$ | | + | |
| rs548234 | 6 | 106,674,727 | 6q21 | *PRDM1* | C/T | 0.40 | 0.34 | 1.30 (1.16–1.44) | $2.3\times10^{-6}$ | + | + | |
| rs2230926 | 6 | 138,237,759 | 6q23 | *TNFAIP3* | G/T | 0.11 | 0.069 | 1.75 (1.47–2.08) | $1.9\times10^{-10}$ | + | + | |
| rs849142 | 7 | 28,152,416 | 7p15 | *JAZF1* | C/T | 0.999 | 0.999 | 2.72 (0.25–29.8) | 0.41 | | + | |
| rs4917014 | 7 | 50,276,409 | 7p12 | *IKZF1* | T/G | 0.58 | 0.53 | 1.24 (1.11–1.38) | $8.1\times10^{-5}$ | | | + |
| rs6964720 | 7 | 75,018,280 | 7q11 | *HIP1* | G/A | 0.25 | 0.19 | 1.43 (1.27–1.62) | $1.3\times10^{-8}$ | | | + |
| rs4728142 | 7 | 128,361,203 | 7q32 | *IRF5* | A/G | 0.16 | 0.11 | 1.48 (1.28–1.72) | $2.4\times10^{-7}$ | + | + | |
| rs2254546 | 8 | 11,381,089 | 8p23 | *BLK* | G/A | 0.78 | 0.72 | 1.42 (1.25–1.61) | $4.1\times10^{-8}$ | + | + | |
| rs1913517 | 10 | 49,789,060 | 10q11 | *WDFY4* | A/G | 0.32 | 0.28 | 1.20 (1.07–1.35) | 0.0013 | | | + |
| rs4963128 | 11 | 579,564 | 11p15 | *KIAA1542* | T/C | 0.98 | 0.97 | 1.58 (1.03–2.44) | 0.038 | + | + | |
| rs2732552 | 11 | 35,041,168 | 11p13 | *PDHX, CD44* | T/C | 0.75 | 0.73 | 1.13 (1.00–1.27) | 0.056 | | + | |
| rs4639966 | 11 | 118,078,729 | 11q23 | Intergenic | T/C | 0.32 | 0.28 | 1.22 (1.09–1.36) | $7.3\times10^{-4}$ | | | + |
| rs6590330 | 11 | 127,816,269 | 11q24 | *ETS1* | A/G | 0.48 | 0.39 | 1.44 (1.30–1.60) | $1.3\times10^{-11}$ | | | + |
| rs1385374 | 12 | 127,866,647 | 12q24 | *SLC15A4* | T/C | 0.19 | 0.16 | 1.21 (1.06–1.38) | 0.0057 | | | + |
| rs7329174 | 13 | 40,456,110 | 13q14 | *ELF1* | G/A | 0.30 | 0.25 | 1.32 (1.18–1.49) | $2.2\times10^{-6}$ | | | + |
| rs7197475 | 16 | 30,550,368 | 16p11 | Intergenic | T/C | 0.12 | 0.10 | 1.20 (1.02–0.41) | 0.031 | | | + |
| rs11150610 | 16 | 31,241,737 | 16p11 | *ITGAM* | C/A | 0.20 | 0.19 | 1.07 (0.94–1.22) | 0.32 | + | + | |
| rs12949531 | 17 | 13,674,531 | 17p12 | Intergenic | T/C | 0.28 | 0.27 | 1.02 (0.91–1.15) | 0.73 | | + | |
| rs463426 | 22 | 20,139,185 | 22q11 | *HIC2,UBE2L3* | T/C | 0.52 | 0.48 | 1.20 (1.08–1.33) | $6.1\times10^{-4}$ | | + | |

[a]Based on forward strand of NCBI Build 36.3.
[b]Defined using gene expression data measured in lymphoblastoid B cell lines [28].
[c]Based on the previously reported studies for SLE susceptibility loci [3–18].
SLE, systemic lupus erythematosus; OR, odds ratio; eQTL, expression quantitative trait locus; GWAS, genome-wide association study.
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## Genotyping and quality control

In GWAS, 946 SLE cases and 3,477 controls were genotyped using Illumina HumanHap610-Quad and Illumina HumanHap550v3 Genotyping BeadChips (Illumina, CA, USA), respectively. After the exclusion of 47 SLE cases and 92 controls with call rates <0.98, SNPs with call rates <0.99 in SLE cases or controls,

**Table 3.** Results of combined study for Japanese patients with SLE.

| rsID | Chr | Position (bp) | Cytoband | Gene | Allele | Stage | No. subjects | | Allele 1 freq. | | OR (95%CI) | P | eQTL[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1/2 | | Case | Control | Case | Control | | | |
| rs340630 | 4 | 88,177,419 | 4q21 | *AFF1* | A/G | GWAS | 891 | 3,383 | 0.56 | 0.51 | 1.22 (1.10–1.36) | $1.5\times10^{-4}$ | + |
| | | | | | | Replication study 1 | 550 | 646 | 0.57 | 0.49 | 1.40 (1.19–1.64) | $4.6\times10^{-5}$ | |
| | | | | | | Replication study 2 | 820 | 27,911 | 0.56 | 0.53 | 1.14 (1.03–1.26) | 0.0094 | |
| | | | | | | Combined study | 2,261 | 31,940 | 0.56 | 0.52 | 1.21 (1.14–1.30) | $8.3\times10^{-9}$ | |

[a]Defined using gene expression data measured in lymphoblastoid B cell lines [28].
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