

contains anchored motifs that bind to HLA-DR B1*0901. In fact, AA213-237 reactive T cells in PBMC of 40% of SS patients produced IFN- γ [23]. We screened 24 altered peptides that are known to inhibit AA213-237 reactive T cell response, as a marker of IFN- γ release *in vitro*. The results demonstrated that VPPGECKQFLSEPT (M3R 223 I \rightarrow K) and VPPGECFIAFLSEPT (M3R 224 Q \rightarrow A) are candidate altered peptide ligands of the second extracellular domain of M3R [23]. These encouraging results suggest that the M3R reactive T cell response can be regulated using APL, such as M3R 223I \rightarrow K and/or M3R 224Q \rightarrow A, representing antigen-specific therapy, in M3R-induced sialadenitis, without any side-effect (e.g., infection or malignancy).

8. Conclusion

M3R reactive T cells were detected in 40% of patients with SS, suggesting that the M3R immune response in SS might function as an autoantigen recognized by autoreactive T cells. Autoantibodies against M3R were identified in around 50% of SS patients and Abs to the 2nd loop of M3R lowered $[Ca^{2+}]_i$, suggesting that certain anti-M3R Abs act as pathogenic Abs. In MIS mice, M3R reactive Th1 and Th17 cells were essential for the development of sialadenitis, suggesting that M3R reactive T cells play a crucial role in the pathogenesis of SS-like autoimmune sialadenitis. Extrapolation of these studies to human suggests the importance of the M3R immune response in the pathogenesis of SS. Furthermore, early evidence suggests the potential of T cell target therapy as well as B cell target in SS.

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ORIGINAL ARTICLE

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

Hiroto Tsuboi^{1,14}, Hiromitsu Asashima¹, Chinatsu Takai¹, Shinya Hagiwara¹, Chihiro Hagiya¹, Masahiro Yokosawa¹, Tomoya Hirota¹, Hisanori Umehara^{2,14}, Atsushi Kawakami^{3,14}, Hideki Nakamura^{3,14}, Hajime Sano^{4,14}, Kazuo Tsubota^{5,14}, Yoko Ogawa^{5,14}, Etsuko Takamura^{6,14}, Ichiro Saito^{7,14}, Hiroko Inoue^{8,14}, Seiji Nakamura^{9,14}, Masafumi Moriyama^{9,14}, Tsutomu Takeuchi^{10,14}, Yoshiya Tanaka^{11,14}, Shintaro Hirata^{11,14}, Tsuneyo Mimori^{12,14}, Hajime Yoshifuji¹², Akiko Ohta^{13,14}, Isao Matsumoto¹, and Takayuki Sumida^{1,14}

¹Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, ²Department of Hematology and Immunology, Kanazawa Medical University, Kanazawa, Ishikawa, Japan, ³Unit of Translational Medicine, Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, ⁴Division of Rheumatology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan, ⁵Department of Ophthalmology, School of Medicine, Keio University, Tokyo, Japan, ⁶Department of Ophthalmology, Tokyo Women's Medical University, School of Medicine, Tokyo, Japan, ⁷Department of Pathology, Tsurumi University School of Dental Medicine, Tsurumi, Japan, ⁸Department of Pharmacology, Nihon Pharmaceutical University, Saitama, Japan, ⁹Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan, ¹⁰Division of Rheumatology, Department of Internal Medicine, School of Medicine, Keio University, Tokyo, Japan, ¹¹The First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan, Kitakyushu, Japan, ¹²Department of Rheumatology and Clinical Immunology, Kyoto University Graduate School of Medicine, Kyoto, Japan, ¹³Department of Public Health, Saitama Medical University Faculty of Medicine, Saitama, Japan, and ¹⁴The Research Team for Autoimmune Diseases, The Research Program for Intractable Disease of the Ministry of Health, Labor and Welfare (MHLW), Tokyo, Japan

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,

Correspondence to: Prof. Takayuki Sumida, Department of Internal Medicine, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-city, Ibaraki 305-8575, Japan. Tel: + 81-29-853-3221. Fax: + 81-29-853-3222. E-mail: tsumida@md.tsukuba.ac.jp

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 ≥ 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].

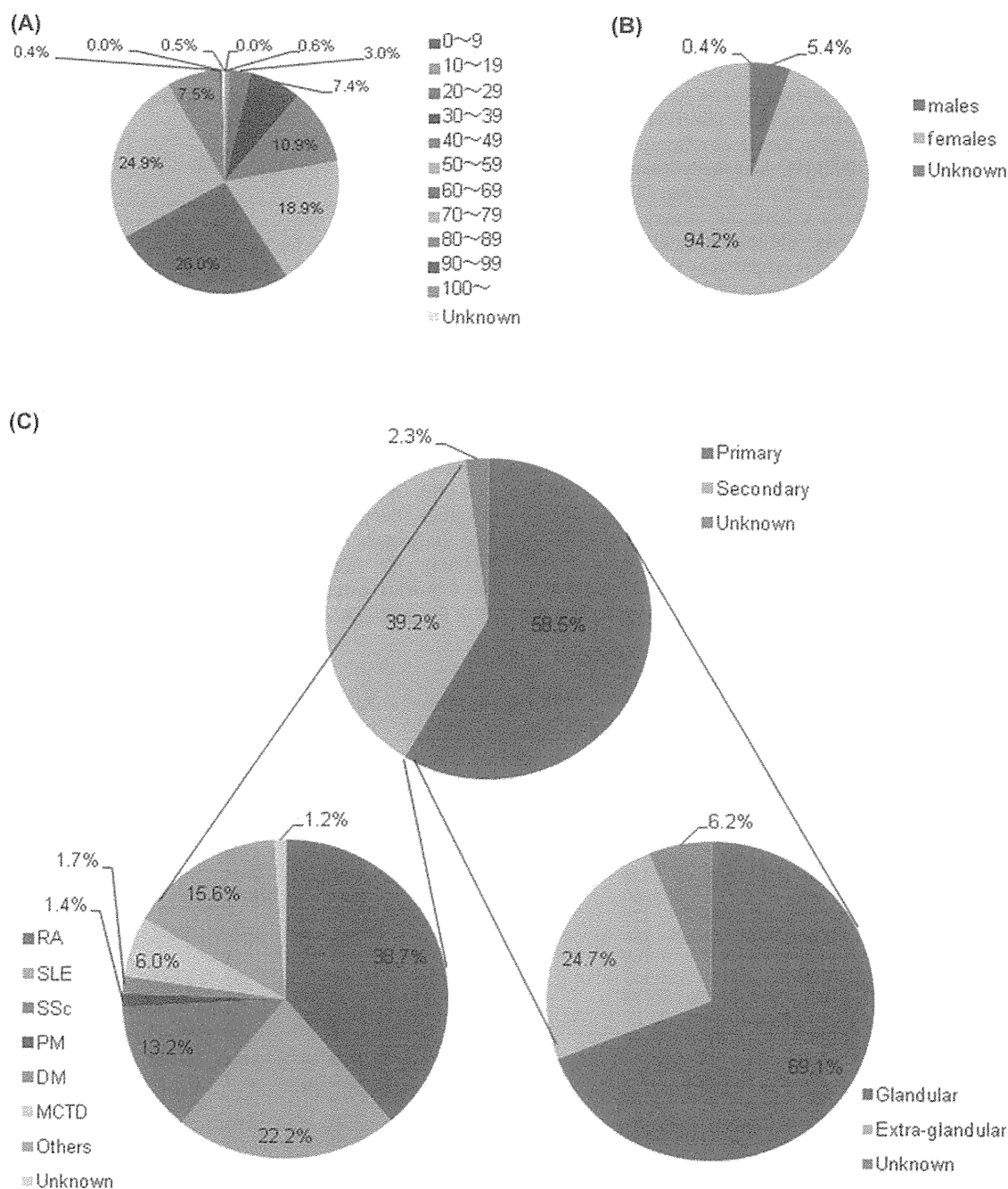


Figure 1. Characteristics of 2,195 patients with SS who participated in the secondary survey. (A). Age distribution; (B). Gender; (C). Disease type. *Top*: frequency of primary and secondary SS. *Bottom right*: frequency of glandular and extra-glandular forms among patients with primary SS. *Bottom left*: 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

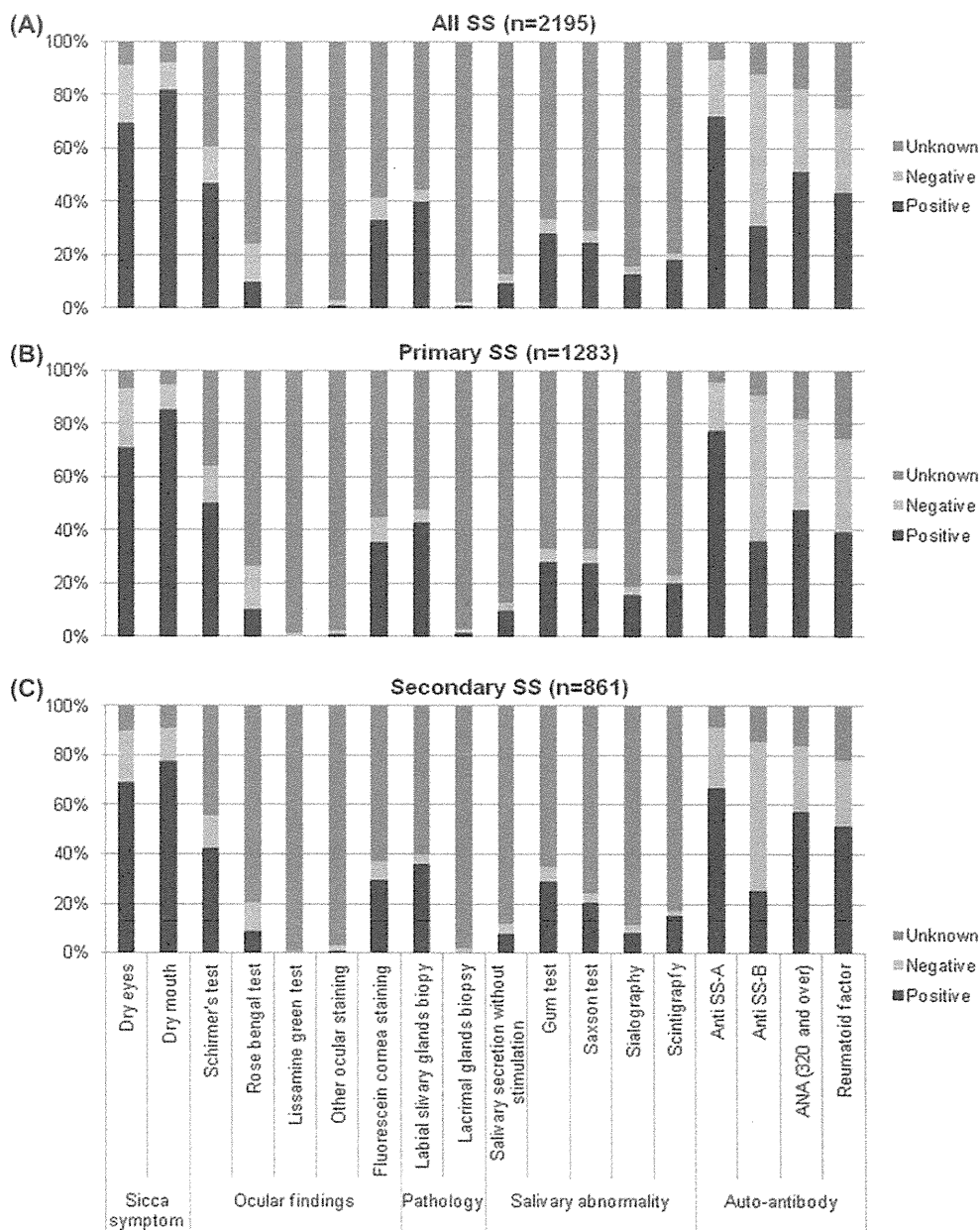


Figure 2. Positivity of various sicca symptoms and objective findings. The histogram shows proportion of patients with positive, negative and unknown (not performed) sicca symptoms and objective findings in (A) all patients with SS, (B) patients with primary SS and (C) patients with secondary SS.

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%



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(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

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).

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).

Figures 4 and 5 summarize the types of treatment according to SS disease type. Corticosteroids were administered 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 administered 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 administered 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

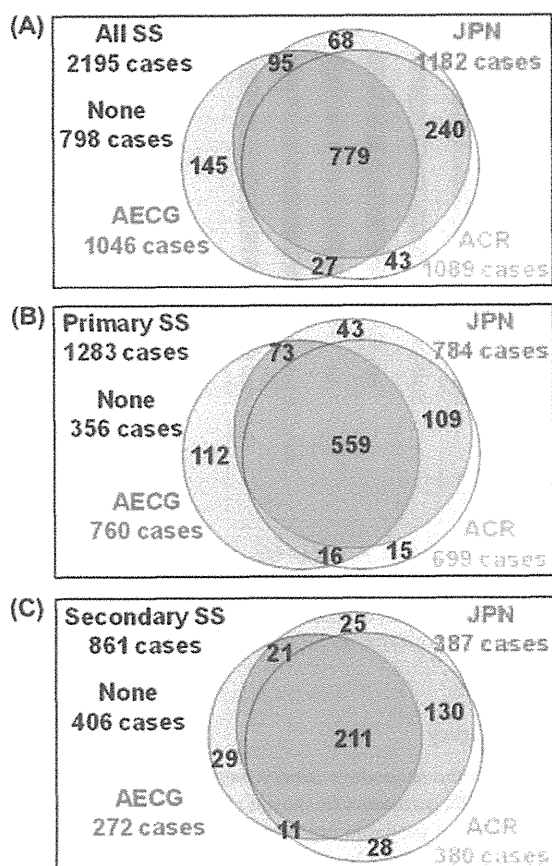


Figure 3. Venn diagrams showing comparison of satisfaction of the three tested criteria sets. (A) Comparison of satisfaction of the three tested criteria sets using data of all ($n = 2,195$) patients with SS. (B) Comparison of satisfaction of the three tested criteria sets using data of 1,283 patients 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).

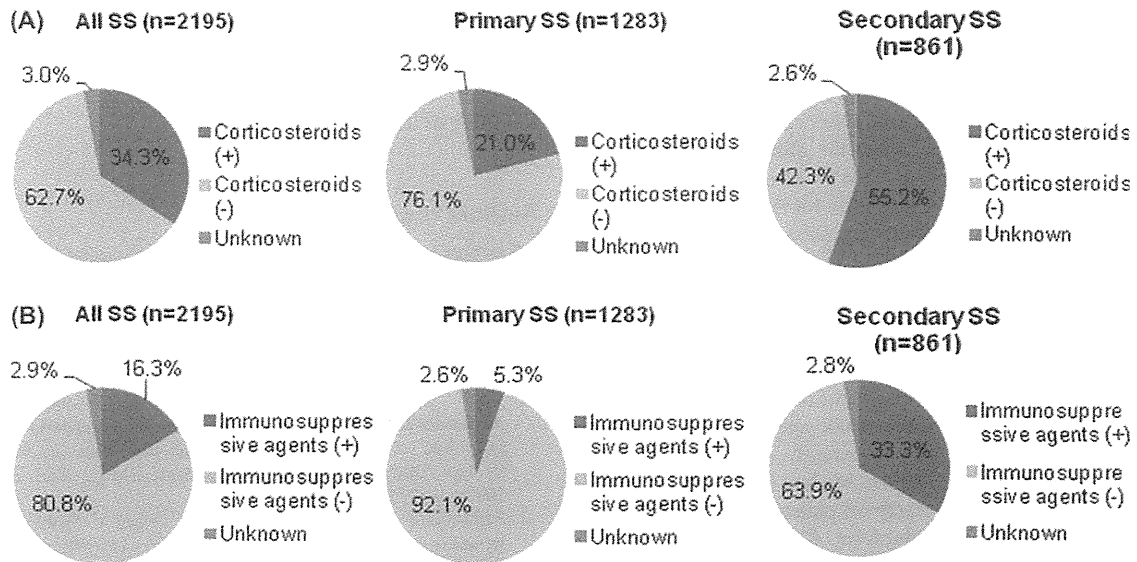


Figure 4. Treatments used in patients with SS and relationship between treatments and disease type (corticosteroids and immunosuppressive agents). (A) Corticosteroids. (B) Immunosuppressive agents. +: patients treated with drugs; -: patients treated without drugs.

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

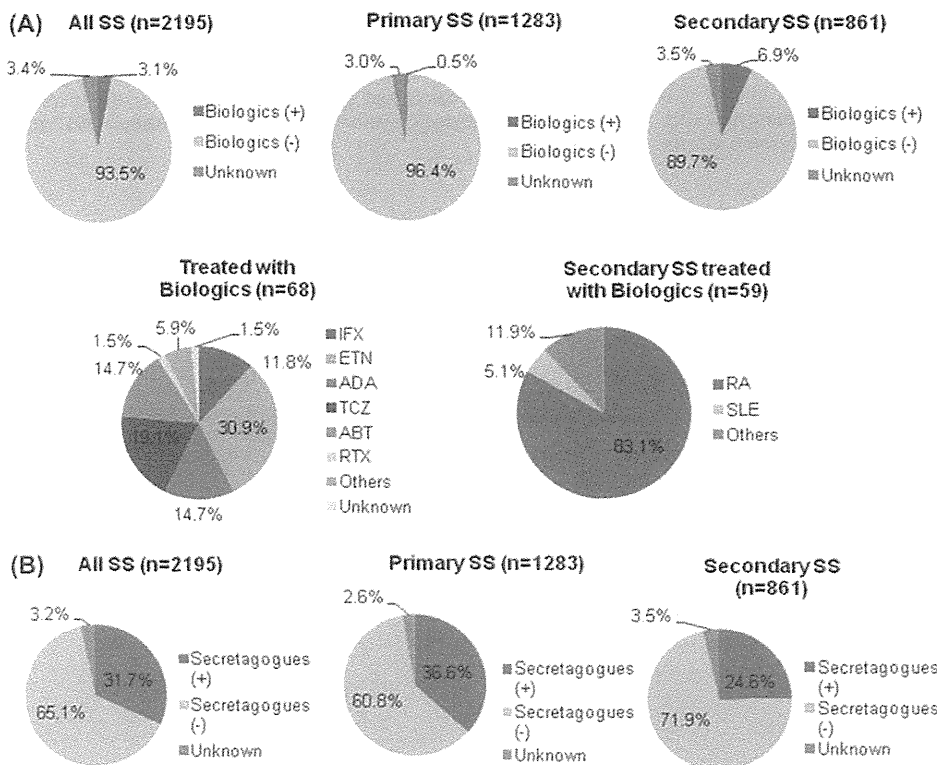


Figure 5. Treatments used in patients with SS and relationship between treatments and disease type (biologics and secretagogues). (A) Biologics. (B) Secretagogues. +, patients treated with drugs; -, patients treated without drugs; IFX, Infliximab; ETN, etanercept; ADA, adalimumab; TCZ, tocilizumab; ABT,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

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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 administered 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.

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Supplementary material available online

Supplementary Table 1-2.

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

Hiroto Tsuboi · Shinya Hagiwara · Hiromitsu Asashima · Hisanori Umehara · Atsushi Kawakami · Hideki Nakamura · Hajime Sano · Kazuo Tsubota · Yoko Ogawa · Etsuko Takamura · Ichiro Saito · Hiroko Inoue · Seiji Nakamura · Masafumi Moriyama · Tsutomu Takeuchi · Yoshiya Tanaka · Shintaro Hirata · Tsuneyo Mimori · Isao Matsumoto · Takayuki Sumida

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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 Auto-immune 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

H. Tsuboi · S. Hagiwara · H. Asashima · I. Matsumoto · T. Sumida (✉)
Department of Internal Medicine, Faculty of Medicine,
University of Tsukuba, 1-1-1 Tennodai, Tsukuba,
Ibaraki 305-8575, Japan
e-mail: tsumida@md.tsukuba.ac.jp

H. Tsuboi · H. Umehara · A. Kawakami · H. Nakamura · H. Sano · K. Tsubota · Y. Ogawa · E. Takamura · I. Saito · H. Inoue · S. Nakamura · M. Moriyama · T. Takeuchi · Y. Tanaka · S. Hirata · T. Mimori · T. Sumida
The Research Team for Autoimmune Diseases,
The Research Program for Intractable Disease of the Ministry
of Health, Labor and Welfare (MHLW), Tokyo, Japan

H. Umehara
Department of Hematology and Immunology,
Kanazawa Medical University, Kanazawa, Japan

A. Kawakami · H. Nakamura
Unit of Translational Medicine, Department of Immunology
and Rheumatology, Nagasaki University Graduate School
of Biomedical Sciences, Nagasaki, Japan

H. Sano
Division of Rheumatology, Department of Internal Medicine,
Hyogo College of Medicine, Nishinomiya, Japan

K. Tsubota · Y. Ogawa
Department of Ophthalmology, School of Medicine,
Keio University, Tokyo, Japan

E. Takamura
Department of Ophthalmology, Tokyo Women's Medical
University, School of Medicine, Tokyo, Japan

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

I. Saito · H. Inoue
Department of Pathology, Tsurumi University School of Dental
Medicine, Yokohama, Kanagawa, Japan

S. Nakamura · M. Moriyama
Section of Oral and Maxillofacial Oncology,
Division of Maxillofacial Diagnostic and Surgical Sciences,
Faculty of Dental Science, Kyushu University, Fukuoka, Japan

T. Takeuchi
Division of Rheumatology, Department of Internal Medicine,
School of Medicine, Keio University, Tokyo, Japan

Y. Tanaka · S. Hirata
The First Department of Internal Medicine, School of Medicine,
University of Occupational and Environmental Health,
Kitakyushu, Japan

T. Mimori
Department of Rheumatology and Clinical Immunology,
Kyoto University Graduate School of Medicine, Kyoto, Japan

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 \geq 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

(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].

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).

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 (≤ 5 mm in 5 min)
 2. Rose bengal score or other ocular dry eye score (≥ 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 ≥ 1 , defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini 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 (≤ 1.5 ml in 15 min)
 2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitory 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
-

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

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)
-

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 ≥ 1 focus/4 mm ²
3. Keratoconjunctivitis sicca with ocular staining score ≥ 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

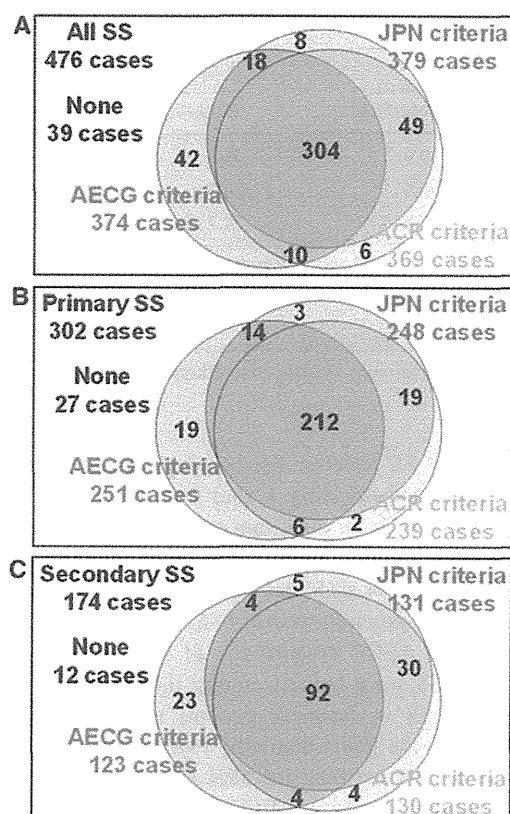


Fig. 1 Venn diagrams showing a comparison of the satisfaction of the three tested systems. **a** Comparison of the satisfaction of the three tested systems, performed using data from all 476 SS patients (302 primary SS and 174 secondary SS). **b** Comparison of the satisfaction of the three tested systems 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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Original article

An association analysis of *HLA-DRB1* with systemic lupus erythematosus and rheumatoid arthritis in a Japanese population: effects of *09:01 allele on disease phenotypes

Kenichi Shimane^{1,2}, Yuta Kochi^{1,2}, Akari Suzuki², Yukinori Okada^{1,2,3}, Tomonori Ishii⁴, Tetsuya Horita⁵, Kazuyoshi Saito⁶, Akiko Okamoto¹, Norihiro Nishimoto⁷, Keiko Myouzen², Michiaki Kubo⁸, Michito Hirakata⁹, Takayuki Sumida¹⁰, Yoshinari Takasaki¹¹, Ryo Yamada^{1,12}, Yusuke Nakamura¹³, Naoyuki Kamatani³ and Kazuhiko Yamamoto^{1,2}

Abstract

Objective. To re-evaluate the roles of *HLA-DRB1* alleles in susceptibility to SLE and RA and their effects on autoantibody status in large-scale Japanese cohorts.

Methods. A total of 656 SLE, 2410 RA and 911 control subjects, who were all Japanese, were genotyped for *HLA-DRB1* alleles using sequence-specific oligonucleotide probes. The association of alleles with disease susceptibility was tested by logistic regression analysis and by the relative predispositional effect method. The association with autoantibody status was examined by the standard χ^2 test.

Results. *HLA-DRB1**15:01, *09:01, *08:02 and *04:01 were significantly associated with SLE susceptibility, while shared epitope (SE) alleles and *DRB1**09:01 were associated with RA susceptibility. The compound heterozygote of *DRB1**09:01/*15:01 conferred an increased risk for SLE compared with the homozygotes for *DRB1**09:01 and *15:01 and was associated with earlier onset of disease, whereas the compound effect of *DRB1*-SE/*09:01 was not clear in RA. *DRB1**09:01 was significantly associated with the appearance of anti-Sm antibody in SLE as well as ACPA in RA, while protectively associated with anti-dsDNA antibody in SLE. No significant interaction was observed between *DRB1**09:01 and smoking status for the appearance of ACPA, unlike that observed in SE alleles in RA.

Conclusion. We identified *HLA-DRB1* alleles associated with SLE and RA in a Japanese population and demonstrated a shared susceptibility of *DRB1**09:01 between the diseases as well as its effect on autoantibody production.

Key words: systemic lupus erythematosus, rheumatoid arthritis, human leukocyte antigen DRB1, autoantibody, genetics, association study, Asian population.

¹Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Tokyo, ²Laboratory for Autoimmune Diseases, Center for Genomic Medicine, Institute of Physical and Chemical Research, Yokohama, ³Laboratory for Statistical Analysis, Center for Genomic Medicine, Institute of Physical and Chemical Research, Yokohama, ⁴Department of Hematology and Rheumatology, Tohoku University Graduate School of Medicine, Sendai, ⁵Department of Medicine II, Hokkaido University Graduate School of Medicine, Sapporo, ⁶First Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, ⁷Laboratory of Immune Regulation, Wakayama Medical University, Wakayama, ⁸Laboratory for Genotyping Development, Center for Genomic Medicine, Institute of Physical and Chemical Research, Yokohama, ⁹Division of Rheumatology, Department of Internal

Medicine, Keio University School of Medicine, Tokyo, ¹⁰Division of Clinical Immunology, Graduate School of Comprehensive Human Science, University of Tsukuba, Tsukuba, ¹¹Department of Internal Medicine and Rheumatology, School of Medicine, Juntendo University, Tokyo, ¹²Center for Genetic Medicine, Kyoto University Graduate School of Medicine, Kyoto and ¹³Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

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Correspondence to: Yuta Kochi, Laboratory for Autoimmune Diseases, Center for Genomic Medicine, Institute of Physical and Chemical Research, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113-8655, Japan.
E-mail: ykochi@src.riken.jp

Introduction

Genome-wide association studies for SLE and RA have discovered many disease susceptibility genes, where the strongest genetic link is seen in the HLA class II region for both diseases [1–3]. In European/Caucasian SLE patients, the HLA-DR3 serotype (or *HLA-DRB1**03:01 for genotype) and DR2 (*DRB1**15:01) have been associated with disease risk [1, 2, 4, 5]. In Asian populations, the association of DR2 was also replicated in many studies, while the exact role of each individual allele comprising the DR2 serotype (*DRB1**15:01 and *15:02 are common in Asians) has not been established [6–10]. In addition, an association of DR9 (*DRB1**09:01), which is rare in European populations, has been implicated in several Asian populations [6–8], although it was not replicated in others [9–11]. This inconsistency may be due to the relatively small sample sizes examined in these individual studies, and it needs to be further tested.

In RA, the disease-associated *DRB1* alleles (DR1/DR4/DR10) are different among European populations but share a conserved amino acid motif (QKRAA/QRRAA/RRRAA) at 70–74, which are now referred to as the shared epitope (SE) [12, 13]. Since ACPA has been recognized in RA, a strong association between SE alleles and the appearance of ACPA has been described [14–16]. Moreover, a gene–environmental interaction between *HLA-DRB1* and cigarette smoking was reported, showing that smokers who carry *DRB1*-SE alleles have a greater risk for the appearance of ACPA [17–19]. In Asian populations, the association of SE alleles has also been repeatedly reported [18, 20]. In addition, *DRB1**09:01, which is not classified as an SE allele, is another risk allele for RA in Asian populations, although the risk is moderate compared with that of SE alleles [18, 20]. The association between the appearance of ACPA and SE alleles has been replicated in Asian populations, but the effect of *DRB1**09:01 on ACPA status is controversial [18, 21–23].

Another issue concerning the association of *HLA-DRB1* alleles with diseases is the genotypic effects, including the gene dosage effect of each allele and the combinational effect of two different alleles. In SLE, compound heterozygotes of susceptible alleles (i.e. DR2/DR3) confer a remarkably increased risk [1]. In European RA patients, compound heterozygotes of SE alleles (i.e. *DRB1**04:01/*04:04) are significantly associated with severe disease in comparison with the homozygotes [24, 25]. In Asia, a study on Koreans demonstrated that the *DRB1**04:05/*09:01 heterozygote conferred a stronger risk for RA than the homozygote for *DRB1**04:05 [26].

Here, to examine and refine the previous findings described so far, we investigated the associations of *HLA-DRB1* alleles with a predisposition to SLE and RA in a Japanese population using a large cohort composed of 656 SLE, 2410 RA and 911 control subjects. We also explored the effect of *DRB1* alleles on disease sub-phenotypes by examining the association between *DRB1* genotypes and autoantibody production in the patients.

Patients and methods

Subjects

The DNA samples of SLE patients [$n = 656$; mean (s.d.) age at the time of enrolment = 43.2 (13.7) years; 89.8% female], RA patients [$n = 2410$; mean (s.d.) age = 60.7 (16.7) years; 81.2% female] and control subjects [$n = 911$; mean (s.d.) age = 52.4 (14.4) years; 25.8% female] were recruited through several medical institutes in Japan under the support of the autoimmune disease study group of Research in Intractable Diseases, Ministry of Health, Labor and Welfare, Japan, for SLE patients, the BioBank Japan Project for RA patients [27] and the Midosuji Rotary Club for the controls. All 656 SLE subjects fulfilled the 1997 revised criteria of the ACR for SLE [28], and the RA subjects met the 1987 revised criteria of ACR for RA [29]. The control subjects were the same as those used in a previous study [30]. Subjects having a history of autoimmune diseases were excluded from control subjects based on a questionnaire. All subjects were Japanese and provided informed consent to participate in the study. The study was approved by the ethical committee of each institute (RIKEN Institute, University of Tokyo, Tohoku University, Hokkaido University, University of Occupational and Environmental Health, Wakayama Medical University, Keio University, University of Tsukuba, Juntendo University and Kyoto University).

Genotyping of *HLA-DRB1*

High-resolution (four-digit) genotyping of *HLA-DRB1* was performed by a sequence-specific PCR method with a WAKFlow HLA typing kit (Wakunaga, Hiroshima, Japan) and a Luminex multi-analyte profiling system (xMAP; Luminex, Austin, TX), according to the manufacturer's instructions. For the analysis of RA patients, *DRB1* alleles that contained amino acid sequences QKRAA/QRRAA/RRRAA at 70–74 were classified as SE+.

Autoantibody and smoking status

Clinical data for 562 SLE subjects (85.7%) were available. All individual ACR criteria were coded as positive, negative or missing. For the study of SLE patients, we obtained autoantibody status from these clinical data, including anti-dsDNA antibody, anti-Sm antibody, aCL and lupus anticoagulant (LAC). The number of subjects available for autoantibody status and the positivity rate for each autoantibody were as follows: anti-dsDNA, $n = 541$, 91.7%; anti-Sm, $n = 451$, 35.5%; anti-CL, $n = 419$, 33.9%; LAC, $n = 349$, 21.2%. All these autoantibodies except for LAC were examined by ELISA. LAC was detected by activated partial thromboplastin time and dilute Russell viper venom time according to the international guideline [31].

Sera from 2384 RA patients (98.9%) were available for the measurement of ACPA. ACPA was measured using the Mesacup CCP test (Medical and Biological Laboratories, Woburn, MA). The positivity rate of ACPA was 79.0%. Smoking status was available for 2294 patients (95.2%) and smoking rates were a total of 32.7%; male

82.2% and female 21.2%. The data were recorded as ever or never smoker. Ever smokers contained former and current smokers.

Statistical analysis

We performed logistic regression analyses in case-control studies to adjust for sex and age. In case-only analyses, we tested the association by the standard χ^2 test using 2×2 contingency tables. Fisher's exact test was applied when a cell value was <5 . Only alleles with frequencies >0.01 in control samples were analysed, and Bonferroni's correction was applied for multiple testing (20 tests, $\alpha=0.0025$). The contingency tables were used to calculate the odds ratio (OR). Disease association was also assessed by the relative predispositional effect (RPE) [32]. When the disease is associated with two or more alleles of a locus, this method identifies the associations sequentially according to their strength. Thus the problem that a strong association with one allele can create misleading deviations in the frequencies of other alleles is alleviated. In the RPE analysis of RA, all the SE-carrying alleles were treated as a single SE allele. We calculated population-attributable risk (PAR) using the following formula: $PAR = f(OR - 1)/(1 + f(OR - 1))$, where f is the allele frequency. The mean age at disease onset was analysed by the Mann-Whitney U test. To evaluate a multiplicative interaction between alleles, we introduced the interaction term in a logistic regression model [19, 33–35]. We also evaluated the interaction by calculating three measures, including the relative excess risk due to interaction (RERI), the proportion attributable to interaction (AP) and the synergy index (S) [36].

We performed k-means cluster analysis to identify cluster groups of SLE patients with similar autoantibody patterns using the clinical data of anti-dsDNA, anti-Sm and anti-CL autoantibodies and LAC, as previously described [37]. We replaced missing data with the mean positive rate of each autoantibody in order to maximize the statistical power [34]. Then we compared autoantibody status and *DRB1* allele frequency among the three groups by χ^2 test using 2×3 tables. All statistical analyses were performed using STATISTICA (StatSoft, Tulsa, OK).

Results

Distribution of *HLA-DRB1* alleles in SLE and RA

The allele distribution of *HLA-DRB1* in SLE, RA and control subjects is presented in Table 1. In SLE, only *DRB1*15:01* showed a significant association with disease susceptibility ($P=4.2 \times 10^{-9}$, OR 2.90). In the RPE analysis, *DRB1*04:01*, **08:02* and **09:01* were significantly associated with SLE susceptibility ($P < 0.0025$). As *DRB1*15:01*, **08:02* and **09:01* were the three prominent alleles in our population showing a strong association with SLE and a substantial extent of PAR (0.117, 0.032 and 0.063, respectively), we focused on the *DRB1*15:01*, **08:02* and **09:01* alleles for further analysis of SLE.

In RA, SE alleles, including *DRB1*04:01* and **04:05*, displayed significant association with disease ($P < 0.0025$), and *DRB1*04:05* showed the highest risk ($P=4.9 \times$

10^{-22} , OR 2.59). When we evaluated SE alleles collectively, they contributed to a substantial proportion of the RA risk when evaluated by PAR (= 0.233). After SE alleles were excluded collectively in the RPE analysis, only *DRB1*09:01* was significantly associated with RA susceptibility ($P=3.8 \times 10^{-8}$). Thereafter we focused on SE alleles and *DRB1*09:01* for further analysis of RA.

Genotypic association analyses of *HLA-DRB1* with disease susceptibility

To analyse the combinational effect of the three major risk alleles of SLE, we grouped the SLE patients by the presence of *DRB1*08:02*, **09:01* and **15:01* alleles (Table 2). When compared with the reference genotype (denoted as X/X in SLE), the homozygote *DRB1*09:01*09:01* showed a greater risk (OR 2.37) than the heterozygote *DRB1*09:01/X* (OR 1.48), which implied the presence of a gene dosage effect. In contrast, no obvious gene dosage effect was seen in *DRB1*15:01*, although a lack of statistical power was undeniable. Strikingly, the compound heterozygote *DRB1*09:01*15:01* conferred the highest risk for SLE (OR 7.42). The heterozygote *DRB1*09:01*15:01* showed a significantly increased risk for SLE in comparison with the homozygote for *DRB1*09:01* ($P=0.0076$, OR 5.05), while no significant difference was observed between the compound heterozygote and the homozygote for *DRB1*15:01* ($P=0.52$, OR 2.13), in which there was a lack of statistical power. When the multiplicative interaction between *DRB1*15:01* and **09:01* was evaluated in a logistic regression model, the coefficient for the interaction term was not significant but marginal ($P=0.078$). However, the three measures for interaction as departures from additivity suggested a possible interaction [RERI 6.17 (95% CI -2.04 , 14.38), AP 0.63 (95% CI 0.31, 0.96), S 3.39 (95% CI 1.22, 9.48)].

In the RA cohort, we grouped the patients by the presence of *DRB1-SE* and **09:01* (Table 2). In comparison with heterozygote SE/Y, homozygote SE/SE showed a significantly increased risk ($P=1.1 \times 10^{-6}$, OR 2.69), which confirmed the gene dosage effect of SE alleles as previously described [24]. The homozygote of *DRB1*09:01* suggested a moderately increased risk for RA compared with the heterozygote ($P=0.093$, OR 1.69). As the risk of compound heterozygote SE/**09:01* did not significantly exceed the risk of homozygote SE/SE nor **09:01*09:01*, there was no dominant compound effect (data not shown). Likewise, no compound effect was detected in **0405*0901* (data not shown), unlike the previous report in a Korean population [26].

In addition to analysing the genotypic effect of *HLA-DRB1* on disease susceptibility, we also analysed its effect on the age of disease onset. Among the genotypes of SLE patients, only the compound heterozygote *DRB1*09:01*15:01* conferred a significantly earlier onset of SLE when compared with X/X (24.3 vs 30.9 years, $P=4.3 \times 10^{-4}$) (Table 3). The same result was obtained when only female patients were analysed. In RA, the homozygote SE/SE and the compound heterozygote SE/**09:01* were significantly associated with an earlier

TABLE 1 Distribution of HLA-DRB1 alleles in 656 SLE patients, 2410 RA patients and 911 control subjects

DRB1 allele ^a	No. of alleles (%)			SLE				RA				SE allele
	SLE cases (n = 1312 alleles)	RA cases (n = 4820 alleles)	Controls (n = 1822 alleles)	Logistic regression		RPE test		Logistic regression		RPE test ^b		
				OR (95% CI)	P-value	P-value	PAR	OR (95% CI)	P-value	P-value	PAR	
*01:01	48 (3.7)	321 (6.7)	106 (5.8)	0.58 (0.36, 0.93)	0.024	-	-	1.14 (0.86, 1.51)	0.35	-	-	+
*04:01	29 (2.2)	117 (2.4)	18 (1.0)	2.93 (1.29, 6.66)	0.010	3.1 × 10 ⁻⁴	0.019	2.51 (1.38, 4.55)	0.0025	-	-	+
*04:03	32 (2.4)	74 (1.5)	62 (3.4)	1.05 (0.57, 1.91)	0.88	-	-	0.47 (0.30, 0.72)	6.9 × 10 ⁻⁴	-	-	-
*04:05	126 (9.6)	1208 (25.1)	235 (12.9)	0.62 (0.47, 0.84)	0.0023	-	-	2.59 (2.14, 6.73)	4.9 × 10 ⁻²²	-	-	+
*04:06	17 (1.3)	116 (2.4)	55 (3.0)	0.50 (0.24, 1.02)	0.058	-	-	0.80 (0.52, 1.23)	0.30	-	-	-
*04:10	27 (2.1)	123 (2.6)	24 (1.3)	0.98 (0.45, 2.12)	0.95	0.011	<0.001	1.90 (1.14, 3.18)	0.014	-	-	+
*08:02	81 (6.2)	123 (2.6)	64 (3.5)	1.95 (1.20, 3.15)	0.0066	3.8 × 10 ⁻⁶	0.032	0.66 (0.44, 0.98)	0.038	-	-	-
*08:03	116 (8.8)	272 (5.6)	164 (9.0)	0.93 (0.67, 1.29)	0.64	-	-	0.61 (0.48, 0.79)	1.8 × 10 ⁻⁴	-	-	-
*09:01	273 (20.8)	827 (17.2)	286 (15.7)	1.43 (1.11, 1.85)	0.0062	2.0 × 10 ⁻⁶	0.063	1.12 (0.94, 1.34)	0.22	3.8 × 10 ⁻⁸	0.018	-
*10:01	4 (0.3)	54 (1.1)	18 (1.0)	0.19 (0.057, 0.65)	0.0082	-	-	1.30 (0.66, 2.57)	0.45	-	-	+
*11:01	20 (1.5)	101 (2.1)	43 (2.4)	0.63 (0.31, 1.28)	0.20	-	-	0.98 (0.60, 1.57)	0.92	-	-	-
*12:01	49 (3.7)	163 (3.4)	72 (4.0)	1.10 (0.66, 1.82)	0.72	-	-	0.92 (0.64, 1.32)	0.65	-	-	-
*12:02	14 (1.1)	58 (1.2)	27 (1.5)	0.83 (0.33, 2.10)	0.70	-	-	0.93 (0.53, 1.66)	0.81	-	-	-
*13:02	45 (3.4)	193 (4.0)	97 (5.3)	0.71 (0.42, 1.19)	0.19	-	-	0.73 (0.53, 1.0)	0.73	-	-	-
*14:03	16 (1.2)	52 (1.1)	20 (1.1)	0.76 (0.33, 1.76)	0.53	-	-	0.78 (0.42, 1.46)	0.44	-	-	-
*14:05	22 (1.7)	53 (1.1)	30 (1.7)	0.95 (0.46, 1.98)	0.89	-	-	0.61 (0.34, 1.07)	0.084	-	-	-
*14:06	11 (0.8)	68 (1.4)	21 (1.2)	0.55 (0.22, 1.39)	0.21	-	-	1.08 (0.60, 1.95)	0.80	-	-	+
*14:54	44 (3.4)	109 (2.3)	48 (2.6)	0.92 (0.53, 1.59)	0.77	0.012	0.002	0.59 (0.39, 0.91)	0.017	-	-	-
*15:01	181 (13.8)	277 (5.8)	127 (7.0)	2.90 (2.03, 4.13)	4.2 × 10 ⁻⁹	2.4 × 10 ⁻¹⁰	0.117	0.89 (0.68, 1.17)	0.41	-	-	-
*15:02	113 (8.6)	414 (8.6)	246 (13.5)	0.60 (0.43, 0.82)	0.0017	-	-	0.54 (0.43, 0.67)	3.7 × 10 ⁻⁸	-	-	-
Other alleles	44 (3.4)	97 (2.0)	59 (3.2)	-	-	-	-	-	-	-	-	-
Total SE+	-	1907 (39.6)	430 (23.6)	-	-	-	-	2.29 (1.96, 2.68)	5.1 × 10 ⁻²⁵	5.4 × 10 ⁻³⁴	0.233	-
Total SE-	-	2913 (60.4)	1392 (76.4)	-	-	-	-	-	-	-	-	-

^aAlleles with frequencies of <0.01 are not listed. ^bSE alleles were excluded collectively in the RPE analysis of RA.