and quantile-quantile plots were drawn using R. We drew regional association plots using the website-based tool LocusZoom, version 1.1 (see URLs; ref. 48). LD plots were generated using Haploview[47] and the UCSC Genome Browser (see URLs).

37. Long, J. *et al.* Genome-wide association study in east Asians identifies novel susceptibility loci for breast cancer. *PLoS Genet.* **8**, e1002532 (2012).
38. Shu, X.O. *et al.* Identification of new genetic risk variants for type 2 diabetes. *PLoS Genet.* **6**, pii: e1001127 (2010).
39. Zheng, W. *et al.* Genetic and clinical predictors for breast cancer risk assessment and stratification among Chinese women. *J. Natl. Cancer Inst.* **102**, 972–981 (2010).
40. Price, A.L. *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* **38**, 904–909 (2006).
41. Sinnott, J.A. & Kraft, P. Artifact due to differential error when cases and controls are imputed from different platforms. *Hum. Genet.* **131**, 111–119 (2012).
42. Jiao, S., Hsu, L., Hutter, C.M. & Peters, U. The use of imputed values in the meta-analysis of genome-wide association studies. *Genet. Epidemiol.* **35**, 597–605 (2011).
43. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
44. Lau, J., Ioannidis, J.P. & Schmid, C.H. Quantitative synthesis in systematic reviews. *Ann. Intern. Med.* **127**, 820–826 (1997).
45. Higgins, J.P. & Thompson, S.G. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* **21**, 1539–1558 (2002).
46. Zhang, B., Beeghly-Fadiel, A., Long, J. & Zheng, W. Genetic variants associated with breast-cancer risk: comprehensive research synopsis, meta-analysis, and epidemiological evidence. *Lancet Oncol.* **12**, 477–488 (2011).
47. Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265 (2005).
48. Pruim, R.J. *et al.* LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* **26**, 2336–2337 (2010).

ARTHRITIS & RHEUMATISM
Vol. 65, No. 2, February 2013, pp 472–480
DOI 10.1002/art.37777

PLD4 as a Novel Susceptibility Gene for Systemic Sclerosis in a Japanese Population

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Objective. **Systemic sclerosis (SSc) is an autoimmune disease for which multiple susceptibility genes have been reported. Genome-wide association studies have shown that large numbers of susceptibility genes are shared among autoimmune diseases. Recently, our group identified 9 novel susceptibility genes associated with rheumatoid arthritis (RA) in a Japanese population. The aim of this study was to elucidate whether the 18 genes that displayed associations or suggestive associations for RA in our previous study are associated with SSc in Japanese.**

Methods. **We performed an association study that included 415 patients with SSc and 16,891 control subjects, followed by a replication study that included 315 patients and 21,054 control subjects. The 18 markers reported to display association with RA were analyzed for their associations with SSc in the first study, and 5 markers were further analyzed in the replication study. The inverse variance method was used to evaluate the associations of these markers with SSc in a combined study.**

Results. **In the phospholipase D4 gene (*PLD4*), rs2841277 displayed a significant association with SSc in Japanese patients ($P = 0.00017$). We observed that rs2841280 in exon 2 of *PLD4* was in strong linkage disequilibrium with rs2841277 and introduced an amino acid alteration. We also observed associations between SSc and rs6932056 in *TNFAIP3* and rs2280381 in *IRF8* ($P = 0.0000095$ and $P = 0.0030$, respectively), both of which displayed associations with SSc in a European population.**

Conclusion. **We determined that *PLD4* is a novel susceptibility gene for SSc in Japanese, thus confirming the involvement of *PLD4* in autoimmunity. Associations between SSc and *TNFAIP3* or *IRF8* were also detected in our Japanese population. SSc and RA appear to share relatively large proportions of their genetic backgrounds.**

Systemic sclerosis (SSc) is a connective tissue disease that affects 7–489 individuals per million worldwide and is characterized by the excess production of extracellular matrix molecules and fibrosis (1). Patients with SSc display skin sclerosis, obliterative microvasculopathy such as Raynaud's phenomenon, and multiorgan involvement. Severe complications of SSc sometimes develop, including interstitial lung disease, pulmonary hypertension, and renal crisis. These severe symptoms

Supported by grants-in-aid from the Ministry of Health, Labor, and Welfare of Japan and from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, by research grants from the Japan Rheumatism Foundation, the Waksman Foundation, and the Mitsubishi Pharma Research Foundation, and by the Genetics and Allied Research in Rheumatic Diseases Networking consortium.

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Submitted for publication June 4, 2012; accepted in revised form October 23, 2012.

and complications of SSc result in a poor prognosis and a shortened lifespan (2,3). No effective method for preventing or curing SSc has been established (4).

It is well known that SSc has genetic components (5); for example, a US study revealed that the incidence of SSc was much higher among the families of patients with SSc compared with the general population (6). Recent technologic developments enabled the use of genome-wide association studies (GWAS) to identify novel susceptibility loci for autoimmune diseases (7). GWAS of European patients with SSc revealed that *CD247* (8), HLA (8), *TNIP1*, *PSORS1C1*, and *RHOB* (9) are susceptibility loci for SSc. In addition, another GWAS identified associations between *IRF8*, *GRB10*, and *SOX5* and limited cutaneous SSc (lcSSc) in a European population (10). Furthermore, studies adopting a candidate gene approach based on subjecting genes to functional inference analysis led to the identification of *STAT4* (11), *IRF5* (12), *TBX21* (13), *NLRP1* (14), *TNFSF4* (15), *CD226* (16), *BLK* (17), and *TNFAIP3* (18) as novel susceptibility genes for SSc in Europeans. SSc association studies in Japanese populations confirmed that *STAT4* (19), *IRF5* (20), and *BLK* (21) are associated with SSc and identified *UBE2L3* as a susceptibility gene for diffuse cutaneous SSc (dcSSc) (22). An association between HLA and SSc was also detected in Asians (23). These findings suggest a clear overlap in the genetic background of SSc between different populations.

It is well known that susceptibility genes are shared by various autoimmune diseases (24). In fact, HLA (25), *STAT4* (26), and *TNFAIP3* (27,28), which are susceptibility genes for SSc, have also been reported to be associated with rheumatoid arthritis (RA). In addition, *PTPN22*, which was shown to be strongly associated with RA in a European population (29), showed a suggestive association with SSc in Europeans (30). The sharing of these susceptibility genes between RA and SSc raises the possibility that newly identified susceptibility genes for RA could also be susceptibility genes for SSc. Recently, a large Japanese consortium, the Genetic and Allied research in Rheumatic diseases Networking consortium, identified 9 novel susceptibility genes and 6 candidate susceptibility genes for RA using a meta-analysis of GWAS and replication studies (31). Four other genes, namely, HLA, *PADI4*, *CCR6*, and *TNFAIP3*, were also confirmed to display associations with RA. Here, we performed a 2-stage association study of Japanese patients with SSc, in which we genotyped these genes as candidate susceptibility loci.

PATIENTS AND METHODS

Study subjects. DNA samples were obtained from 415 patients with SSc at Kyoto University Hospital and Tokyo Women's Medical University; these samples comprised the first set. Independent DNA samples were obtained from 315 patients with SSc at Keio University Hospital, Sagamihara National Hospital, and Kanazawa University Hospital; these samples were used as the replication set. All patients were Japanese, all had a diagnosis of SSc as determined by a rheumatologist, and all fulfilled the 1980 American College of Rheumatology classification criteria for SSc (32). The patients with SSc for whom clinical information was available were classified as having lcSSc or dcSSc, according to the definitions developed by LeRoy et al (33). The control samples were described in detail in our previous study (31). The current study was approved by the local ethics committees at each institution, and written informed consent was obtained from all subjects. The basic characteristics of the study subjects are shown in Table 1.

Genotyping. The 9 novel susceptibility markers, 6 potentially associated markers, and 4 confirmed markers of RA that were identified in our previous study in a Japanese population (31) were chosen as candidate susceptibility markers for SSc in Japanese. Eighteen of the 19 markers (HLA was excluded; see Results), none of which had previously been reported to be associated with SSc in Japanese individuals, were genotyped in the current study. The 5 candidate markers in the first set that showed associations with P values less than 0.1 were further genotyped in the replication study. Single-nucleotide polymorphisms (SNPs) rs2841280 and rs894037 were chosen as candidate causative variants in the phospholipase D4 gene (*PLD4*) region. Because rs894037 was shown to be monomorphic in Japanese, rs2841280 was genotyped in 334 control subjects, in addition to all patients, for imputation reference. The patients in the first and replication studies were genotyped at Kyoto University or Tokyo Women's Medical University and at Keio University or University of Tsukuba, respectively, using TaqMan assays (Applied Biosystems). The genotyping methods in control subjects were described in detail in our previous study (31).

Briefly, control genotypes in the first set were imputed based on the genome-scanning data, using mach2dat software with HapMap Phase II East Asian Populations as reference. The control genotypes for the replication study were extracted from genome-scanning data for the markers included on Illumina HumanHap610 Quad BeadChips. The genotypes for rs6932056 (which is not included in the array) were imputed based on the genome-scanning data, using mach2dat software with HapMap Phase II East Asian Populations as reference, and were used as control data for the replication set. The genotypes for rs2841280 (which is not included in the HapMap data or the array) were also imputed in control subjects, based on the genome-scanning data, using mach2dat software. Genotyping data for the 334 control subjects as determined by TaqMan assay in combination with genome-scanning data were used as reference.

Statistical analysis. The associations between the genotyped markers and SSc were analyzed using a Cochran-Armitage trend test in both the first and replication studies. Subanalyses were performed by comparing the genotypes of

Table 1. Characteristics of the study population*

	Patients	Controls
First set		
Institutions	Kyoto University, Tokyo Women's Medical University	Kyoto University, Tokyo Women's Medical University, BioBank Japan
Typing	TaqMan assay	Illumina HumanHap610 Quad BeadChip, Illumina HumanHap550 BeadChip, Affymetrix Genome-Wide Human SNP Array 6.0
Limited SSc/diffuse SSc, %	49.6/50.4	Not applicable
Anti–topo I/ACA, %	30.6/31.1	Not applicable
Interstitial lung disease, %	48.9	Not applicable
Age, mean ± SD years	50.9 ± 14.7	60.9 ± 12.5
Female, %	91.3	44.9
Replication set		
Institutions	Keio University, Sagamihara National Hospital, Kanazawa University	Kyoto University, BioBank Japan
Typing	TaqMan assay	Illumina HumanHap550 BeadChip, Illumina HumanHap610 Quad BeadChip
Limited SSc/diffuse SSc, %	63.8/34.6	Not applicable
Anti–topo I/ACA, %	29.5/35.2	Not applicable
Interstitial lung disease, %	43.2	Not applicable
Age, mean ± SD years	51.4 ± 14.1	59.3 ± 14.2
Female, %	87.3	48.4

* The first set included 415 patients with systemic sclerosis (SSc) and 16,891 control subjects. The replication set included 315 patients with SSc and 21,054 control subjects. Anti–topo I = anti–topoisomerase I; ACA = anticentromere antibody.

the control subjects with those of patients in the SSc subgroups based on the disease phenotypes. The subanalyses used the same control subjects as were used in the association studies. Intracase analyses based on phenotypes were also performed.

Odds ratios (ORs) and 95% confidence intervals were also calculated. The associations detected in the first and replication studies were then meta-analyzed using the inverse variance method. The resultant *P* values were corrected using the Benjamini-Hochberg false discovery rate (FDR) criterion, and corrected *P* values less than 0.05 were regarded as significant in both the combined study and the subanalyses. The efficiency of the current study was estimated by calculating the likelihood of detecting 3 significant markers (after correcting the *P* values using the FDR method) among 18 randomly selected markers. After the statistically significant markers were identified, the best-fit model for each association was analyzed using dominant, recessive, trend, and allelic chi-square tests or models. Statistical analyses were performed using R or SPSS (version 18) software.

RESULTS

Analyses of candidate genes for SSc in a Japanese population. The 415 patients with SSc and 16,891 control subjects in the first set were genotyped for the 18 markers that were shown to have associations or suspected associations with RA in our previous study. The HLA region was excluded from the genotyped markers, because this region has already been shown to be associated with SSc in Asians. The allele frequencies of the patients were compared with those of the control subjects, using a Cochran-Armitage trend test.

As a result, 3 markers that demonstrated associations with *P* values less than 0.01 in the first set (Table 2) were identified, namely, rs6932056 in the *TNFAIP3* region ($P = 0.0000038$, OR 1.69), rs10821944 in the *ARID5B* region ($P = 0.0025$, OR 1.25), and rs2841277 in the *PLD4* region ($P = 0.0054$, OR 1.25). Two loci that showed suggestive associations with *P* values less than 0.1 (Table 2) were also identified, namely, rs12529514 in the *CD83* region ($P = 0.083$, OR 1.18) and rs2280381 in the *IRF8* region ($P = 0.095$, OR 1.19). The *TNFAIP3* and *IRF8* regions were previously reported to display associations with SSc and lcSSc, respectively, in European populations (10,18). These 5 markers were selected as candidate susceptibility markers for SSc in Japanese and were subjected to validation.

Next, a replication study consisting of 315 patients with SSc and 21,054 control subjects was performed to validate the associations of the 5 markers with SSc. The patients were genotyped for the 5 markers. The genotypes of the control subjects for the 5 markers, except rs6932056, were extracted from the Illumina Infinium HumanHap610 Quad array, as reported previously (31). The genotypes for rs6932056 were imputed based on genome-scanning data using mach2dat soft-

Table 2. Association studies of Japanese patients with SSc*

				Allele 1 frequency								
				First set			Replication set			Combined study		
SNP	Chr	Gene	Allele 1/2	Controls	Patients	*P*	Controls†	Patients	*P*	*P*, patients vs. controls	OR (95% CI)	*P*, patients without overlapping RA vs. controls
rs766449	1	*PADI4*	T/C	0.40	0.37	0.12	–	–	–	–	–	–
rs11900673	2	*B3GNT2*	T/C	0.29	0.28	0.65	–	–	–	–	–	–
rs2867461	4	*ANXA3*	A/G	0.44	0.43	0.57	–	–	–	–	–	–
rs657075	5	*IL3-CSF2*	A/G	0.36	0.34	0.25	–	–	–	–	–	–
rs12529514	6	*CD83*	C/T	0.14	0.16	0.083	0.15	0.16	0.31	0.046	1.15 (1.00–1.33)	0.040
rs1571878	6	*CCR6*	C/T	0.49	0.47	0.28	–	–	–	–	–	–
rs6932056	6	*TNFAIP3*	C/T	0.069	0.11	3.8×10^{-6}	0.067	0.079	0.23	9.5×10^{-6}	1.50 (1.25–1.80)	5.4×10^{-6}
rs2233434	6	*NFKBIE*	G/A	0.21	0.21	0.93	–	–	–	–	–	–
rs10821944	10	*ARID5B*	G/T	0.36	0.41	0.0025	0.36	0.37	0.64	0.0073	1.16 (1.04–1.29)	0.010
rs3781913	11	*PDE2A-CENTD2*	T/G	0.69	0.69	0.91	–	–	–	–	–	–
rs4937362	11	*ETS1-FLI1*	T/C	0.68	0.68	0.88	–	–	–	–	–	–
rs2841277	14	*PLD4*	T/C	0.69	0.74	0.0054	0.69	0.73	0.012	0.00017	1.25 (1.11–1.41)	0.00052
rs3783637	14	*GCH1*	C/T	0.74	0.73	0.54	–	–	–	–	–	–
rs1957895	14	*PRKCH*	G/T	0.39	0.41	0.26	–	–	–	–	–	–
rs6496667	15	*ZNF774*	A/C	0.35	0.37	0.33	–	–	–	–	–	–
rs7404928	16	*PRKCB1*	T/C	0.62	0.63	0.51	–	–	–	–	–	–
rs2280381	16	*IRF8*	T/C	0.84	0.86	0.095	0.83	0.87	0.0099	0.0030	1.26 (1.08–1.47)	0.0021
rs2847297	18	*PTPN2*	G/A	0.34	0.34	0.85	–	–	–	–	–	–

* SSc = systemic sclerosis; SNP = single-nucleotide polymorphism; Chr = chromosome; OR = odds ratio; 95% CI = 95% confidence interval; RA = rheumatoid arthritis.
† The control rs6932056 genotypes used in the replication study were imputed using genome-scanning data obtained for 3,765 subjects.

ware, because rs6932056 was not included in the array. As a result, rs2841277 in the *PLD4* region and rs2280381 in the *IRF8* region showed relatively strong associations with SSc ($P = 0.012$, OR 1.25 and $P = 0.0099$, OR 1.37, respectively) (Table 2). Interestingly, we observed that all 5 of the markers that displayed associations in the first study also demonstrated the same association directions in the replication study.

The inverse variance method was used to combine the data for the first and replication studies. SNPs rs2841277 in the *PLD4* region, rs6932056 in the *TNFAIP3* region, and rs2280381 in the *IRF8* region showed significant associations with SSc even after correcting the associated *P* values using the FDR method for multiple testing (Table 2). Importantly, all 3 of these loci shared risk alleles with RA. Although rs6932056 in the *TNFAIP3* region did not show a strong association with SSc in the replication study, its association was significant in the combined study. The *PLD4* region was shown to be a novel susceptibility gene for SSc, and, for the first time, the *TNFAIP3* and *IRF8* regions were confirmed to be associated with SSc in Japanese.

The association between rs2841277 and SSc was then investigated in detail. When the 200-kbp region around rs2841277 was evaluated, 2 hypothetical genes and cell division cycle associated 4 gene (*CDCA4*) were located at the region, in addition to *PLD4*. *PLD4* was the only gene whose region showed moderate to strong linkage disequilibrium (LD) with rs2841277, indicating *PLD4* as a susceptibility gene (Figure 1A). We vigorously searched candidate markers in exons of *PLD4* that showed strong LD with rs2841277 and selected 2 markers registered in the 1000 Genomes Project (34) that displayed >5% frequency in genotyped subjects, namely, rs2841280 (Figure 1B) and rs894037 in exon 2. Genotyping of these polymorphisms revealed strong LD between rs2841280 (E27Q) and rs2841277 ($D' = 0.98$, $r^2 = 0.75$) and monomorphism of rs894037 in Japanese. An association study of rs2841280 using control genotypes obtained by imputation supported association of *PLD4* with SSc ($P = 6.3 \times 10^{-5}$) (see Supplementary Tables 1 and 2, available on the *Arthritis & Rheumatism* web site at http://onlinelibrary.wiley.com/doi/10.002/art.37777/abstract).

Because the 3 loci were associated with RA in a Japanese population, we analyzed whether the associations with SSc in the current study were contributed by patients with both RA and SSc. When 22 patients who had RA as well as SSc were excluded, significant associations for the 3 loci were still observed (Table 2). A

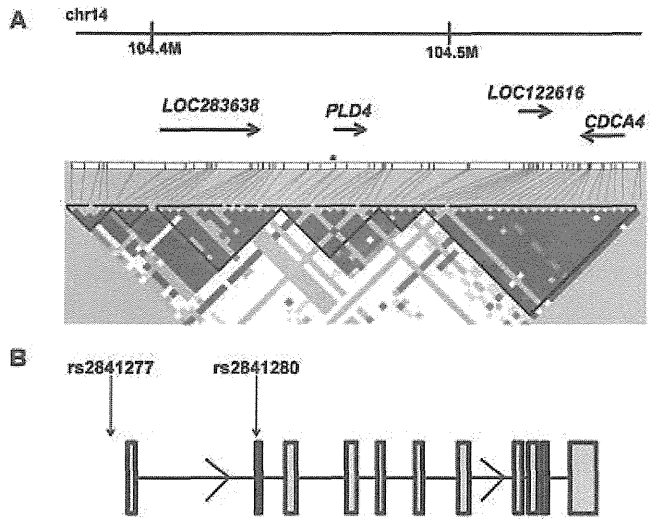

Figure 1. Linkage disequilibrium (LD) block around the *PLD4* region and the *PLD4* structure. **A,** LD block and genes around *PLD4*. The LD block is based on HapMap phase 3 data. **Asterisk** indicates rs2841277. **B,** Schematic view of *PLD4* structure. Rectangles represent exons of *PLD4*.

further stringent analysis excluding patients with other autoimmune diseases demonstrated significant associations of the 3 genes (see Supplementary Table 2). When we compared SSc patients with and those without other autoimmune diseases for the associated alleles, no differences were observed (data not shown).

Subanalysis of types of SSc. Previous studies have revealed that the genetic background of SSc varies between different types of SSc (11,18). Thus, subanalyses of the 5 regions examined in the combined study were performed, in which the allele frequencies of the control subjects were compared with those of the patients with lcSSc or dcSSc. The control subjects were the same as those used in the first study or the combined study. Although *PLD4* and *TNFAIP3* did not display a preference for either SSc phenotype, *IRF8* and *ARID5* showed suggestive preferences for lcSSc, and *CD83* showed a suggestive preference for dcSSc (Table 3).

We also investigated whether the susceptibility loci affect autoantibody status and severe complications. The association studies revealed an association of *TNFAIP3* with SSc patients who possess anticentromere antibodies (ACAs) (see Supplementary Table 3, available on the *Arthritis & Rheumatism* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.37777/abstract), but intracase analyses did not demonstrate clear significance ($P = 0.043$). We did not observe other associations between the susceptibility loci and clinical phenotypes of SSc, in either case–control analyses or intracase analyses.

Efficacy of the current study. In the current study, a candidate gene analysis was performed based on a meta-analysis of RA GWAS, because many susceptibility genes for autoimmune disease have been reported

Table 3. Associations of the 2 SSc subtypes*

SNP	Chr	Gene	Allele 1/2	Controls, allele 1 frequency	Limited cutaneous SSc (n = 408)			Diffuse cutaneous SSc (n = 318)		
					Allele 1 frequency	*P*	OR (95% CI)	Allele 1 frequency	*P*	OR (95% CI)
rs766449	1	*PADI4*	T/C	0.40	0.39	0.52	0.94 (0.77–1.14)	0.36	0.11	0.85 (0.69–1.04)
rs11900673	2	*B3GNT2*	T/C	0.29	0.25	0.096	0.82 (0.66–1.03)	0.31	0.32	1.11 (0.9–1.38)
rs2867461	4	*ANXA3*	A/G	0.44	0.42	0.40	0.92 (0.75–1.12)	0.44	0.97	1.00 (0.82–1.22)
rs657075	5	*IL3-CSF2*	A/G	0.36	0.34	0.54	0.94 (0.76–1.15)	0.33	0.23	0.88 (0.72–1.08)
rs12529514	6	*CD83*	C/T	0.14	0.15	0.79	1.03 (0.85–1.25)	0.18	0.0075	1.32 (1.08–1.62)
rs1571878	6	*CCR6*	C/T	0.49	0.48	0.81	0.98 (0.80–1.19)	0.46	0.20	0.88 (0.72–1.07)
rs6932056	6	*TNFAIP3*	C/T	0.069	0.093	0.0062	1.40 (1.1–1.78)	0.10	0.00063	1.57 (1.21–2.04)
rs2233434	6	*NFKBIE*	G/A	0.21	0.20	0.60	0.94 (0.73–1.20)	0.22	0.70	1.05 (0.83–1.33)
rs10821944	10	*ARID5B*	G/T	0.36	0.40	0.0085	1.22 (1.05–1.41)	0.38	0.30	1.09 (0.93–1.29)
rs3781913	11	*PDE2A-CENTD2*	T/G	0.69	0.69	0.98	1.00 (0.81–1.24)	0.69	0.90	1.01 (0.82–1.25)
rs2841277	14	*PLD4*	T/C	0.69	0.73	0.0067	1.24 (1.06–1.45)	0.74	0.0049	1.29 (1.08–1.55)
rs2841280	14	*PLD4*	C/G	0.64	0.69	0.0011	1.30 (1.11–1.52)	0.69	0.0086	1.27 (1.06–1.51)
rs2847297	18	*PTPN2*	G/A	0.34	0.33	0.67	0.96 (0.78–1.18)	0.34	0.87	1.02 (0.83–1.25)
rs4937362	11	*ETS1-FLI1*	T/C	0.68	0.68	0.75	0.97 (0.78–1.19)	0.69	0.92	1.01 (0.82–1.25)
rs3783637	14	*GCH1*	C/T	0.74	0.73	0.69	0.96 (0.77–1.19)	0.73	0.65	0.95 (0.76–1.18)
rs1957895	14	*PRKCH*	G/T	0.39	0.40	0.84	1.02 (0.84–1.25)	0.42	0.16	1.15 (0.95–1.41)
rs6496667	15	*ZNF774*	A/C	0.35	0.39	0.088	1.19 (0.97–1.45)	0.34	0.75	0.97 (0.79–1.19)
rs7404928	16	*PRKCB1*	T/C	0.62	0.61	0.60	0.95 (0.78–1.16)	0.66	0.15	1.17 (0.95–1.44)
rs2280381	16	*IRF8*	T/C	0.84	0.88	0.0038	1.36 (1.11–1.68)	0.86	0.21	1.16 (0.92–1.45)

* SSc = systemic sclerosis; SNP = single-nucleotide polymorphism; Chr = chromosome; OR = odds ratio; 95% CI = 95% confidence interval.

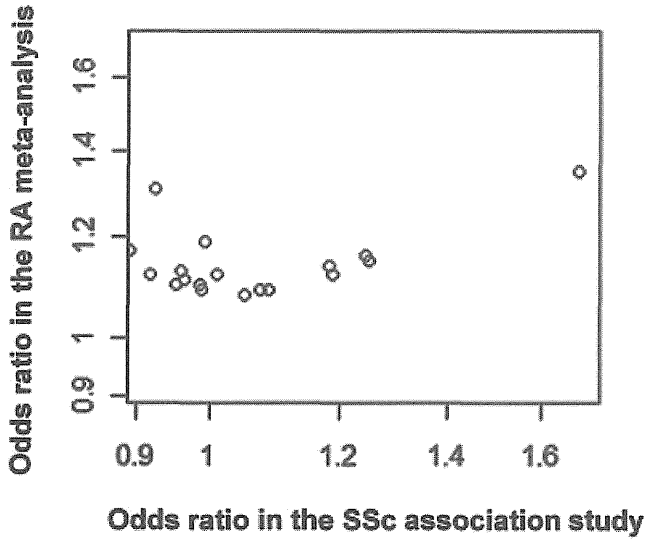

Figure 2. Comparison of associations for systemic sclerosis (SSc) and rheumatoid arthritis (RA). The odds ratios obtained for 18 genes in association studies of SSc and RA are plotted.

to be shared by a wide range of diseases. As a result, 3 susceptibility genes for SSc in Japanese were identified. Thus, we analyzed whether the candidate gene approach taken in the current study for detecting novel susceptibility genes for SSc was effective. When the likelihood of finding 3 susceptibility genes among 18 genes by chance was calculated, the likelihood was determined to be 2.5×10^{-8}. These results indicated that our approach to identifying novel susceptibility genes for systemic diseases is effective. It would be interesting to compare the risk direction of the genotyped markers between RA and SSc. Although the 3 susceptibility loci for SSc shared risk direction with RA, no correspondence of the risk directions of the markers between the 2 diseases was detected (Figure 2). This indicated that a large proportion of the 18 RA markers are not shared by SSc, and that the lack of association between the 13 markers and SSc was not attributable to the low power produced by the relatively small number of SSc patients included in this study.

DISCUSSION

Because SSc can lead to severe complications, poor quality of life, and shortened survival, clarifying the characteristics of SSc is important. Clarification of the disease would aid the search for novel therapeutic targets and the development of new therapeutic strategies. Detecting susceptibility genes using GWAS or a candidate gene approach would also help to uncover the pathophysiology underlying SSc.

Previous studies have revealed that more than 15 markers and loci are associated with SSc. However, the markers detected so far cannot fully explain the genetics of SSc, indicating that many susceptibility genes are yet to be identified. Because a relatively large proportion of RA susceptibility genes are shared by other autoimmune diseases (24), a candidate gene approach using novel markers observed in GWAS of RA is a fascinating way of identifying new SSc markers. In fact, some of the novel susceptibility markers for RA identified in the meta-analysis were shown to be susceptibility markers for systemic lupus erythematosus (SLE) and Graves' disease (31).

In the current study, we successfully identified 3 susceptibility genes for SSc in Japanese. No studies have identified *PLD4* as an SSc-associated locus. The current study is also the first to detect *TNFAIP3* and *IRF8* as susceptibility genes for SSc in a Japanese population. The best-fit models for each association are shown in Supplementary Table 4, available on the *Arthritis & Rheumatism* web site at http://onlinelibrary.wiley.com/doi/10.002/art.37777/abstract.

It is conceivable that these 3 associations might have been obtained due to the overlap of RA and SSc. Even after excluding the patients with both RA and SSc based on physicians' reports, the significant associations for the 3 loci were still observed (Table 3). Information regarding rheumatoid factor (RF) and anti–citrullinated protein antibody (ACPA) was available for 371 SSc patients without RA and 65 SSc patients without RA, respectively, of whom 21.6% and 10.8% were positive for RF and ACPA, respectively. These prevalences are compatible with those previously observed in SSc patients without RA (35,36). Moreover, we showed that the effect sizes and risk direction of the markers tested in this study were dissociated between SSc and RA. In addition, further stringent analysis comprising SSc patients without any autoimmune disease also showed the associations of the 3 loci. These results indicate that the associations of the 3 loci are not attributable to overlapping of RA or other diseases.

Although the associations of the *ARID5B* and *CD83* loci with SSc did not reach a stringently significant level in the combined study, the tendencies toward an association with SSc displayed by rs10821944 in the *ARID5B* locus and rs12529514 in the *CD83* region in the first study were maintained in the replication study. This indicates that these loci are potential susceptibility regions for SSc. Further replication studies are needed to

address the associations of these 2 loci with SSc in a Japanese population.

Because *TNFAIP3* was reported to be strongly associated with SSc in a European population (18), the significant associations detected in the combined study indicate that *TNFAIP3* displays general associations with SSc that go beyond ethnic boundaries. In addition, rs6932056, which displayed a strong association with SSc in a European population (18), is in strong LD with rs5029939 ($r^2 = 0.85$) in the Japanese population. SNP rs6932056 also displays strong LD with rs2230926, a missense mutation of *TNFAIP3* ($r^2 = 0.85$), in Japanese. The rs2230926 missense mutation leads to an amino acid alteration in the OTU (ovarian tumor) domain of the A20 protein, which is considered to result in decreased NF-κB signaling. Because we did not observe strong associations between rs6932056 and SSc in the replication study, it will be necessary to reexamine the association between *TNFAIP3* and SSc using independent sample sets of Japanese patients with SSc, in spite of the significant associations detected in this study.

PLD4 is a recently reported member of the phospholipase family without phospholipase D activity. *PLD4* is expressed in the spleen and early postnatal microglia in the white matter of mice (37). The phenotypes of *Pld4*-deficient mice have not been reported. In addition, little is known about the expression or distribution of *PLD4* in humans. Although the functions of *PLD4* are also poorly understood, it is known to be involved in the phagocytosis of microglia (38). The expression of *PLD4* around the marginal zone in the spleen might support the functional involvement of *PLD4* in immunologic systems. It is interesting that rs2841280, which alters an amino acid of PLD-4, is associated with SSc. Minor allele G of rs2841280 is associated in a protective manner. The impact of an amino acid alteration brought by rs2841280 on the effect of PLD-4 protein is not known.

When we analyzed the impact of the amino acid alteration using in silico analysis (SIFT software; http://sift.jcvi.org/), it was shown to result in a small effect. However, the association raises the possibility that this polymorphism leads functional modulation of PLD-4, and it is feasible to analyze the functional change of PLD-4 protein with rs2841280, using animal models of SSc. When we performed an in silico analysis of the effect of rs2841277 and rs2841280 on *PLD4* expression, we did not detect any clear associations between the 2 genotypes and *PLD4* transcription ($P > 0.05$) (39). Therefore, in spite of the association of these 2 mutations, it has not been confirmed whether one of these 2 polymorphisms is the causative mutation.

Although the detection of a P value less than 5×10^{-8} in a GWAS is stringent evidence of an association between a marker and a particular disease, the detection of suggestive associations between the *PLD4* region and SSc in European GWAS would indicate that associations exist between *PLD4* and SSc in other populations. However, when we examined the associations between the *PLD4* locus or nearby loci and SSc in GWAS involving a European population, we did not detect any strong associations ($P < 10^{-4}$) (8,9). According to the HapMap database, the European population displays a higher risk allele frequency for rs2841277 than the Japanese population. In addition, the HapMap database also indicates that the LD block spanning *PLD4*, which includes rs2841277, is similar in Europeans and Japanese. Nevertheless, a European population did not show a strong association between *PLD4* and SSc, suggesting that *PLD4* has a stronger effect on autoimmune diseases in Japanese than in Europeans. There is also a possibility that these 2 polymorphisms are only markers, and that a rare variant in LD with the 2 markers affects disease onset. A rare causative variant might explain a different association of *PLD4* with SSc between populations.

IRF8 was shown to be associated with SLE in a European population (40). Interferon regulatory factor 8 (IRF-8) protein is a transcription factor involved in the interferon pathway. The interferon pathway has been shown to be involved with a broad range of autoimmune diseases, including SSc (41). Thus, it is interesting that *IRF5* and *IRF8*, both of which belong to the IRF family, displayed associations with SSc. Although a European GWAS of SSc patients revealed suggestive associations between the *IRF4* locus and SSc, the results were not successfully replicated (8), indicating that the different functional roles of each IRF family molecule might influence the development of SSc. *IRF8* promotes B cell differentiation; however, the roles and importance of B cells in skin fibrosis in SSc patients have not been established (42–44). *IRF8* and its mutant variants are also known to be involved in the development of dendritic cells (45). Thus, the association between *IRF8* and SSc might indicate the involvement of B cells and dendritic cells in the development of SSc.

When the patients with SSc were classified as having either lcSSc or dcSSc and subanalyses were performed, *ARID5B*, *IRF8*, and *CD83* displayed stronger associations with one of the 2 phenotypes. However, the associations of these 3 markers with the phenotypes

were not strong enough to provide convincing evidence of a clear distinction between the genetic backgrounds of the 2 SSc phenotypes. When the associations of the SSc subtypes with the other 13 markers in the first set were analyzed, no strong association was detected ($P > 0.05$). Other subanalyses of the susceptibility loci in the combined set did not show significant results between disease phenotypes, due to lack of power. Because classification according to disease phenotypes resulted in limited numbers of subjects in each subset, we conducted this subanalysis only in the combined set. The association between *TNFAIP3* and ACAs should be confirmed in a large-scale association study.

Although GWAS are an extremely powerful way to detect novel susceptibility genes for diseases, GWAS of patients with SSc have been performed only in European populations. Our study detected strong evidence for the sharing of susceptibility genes between RA and SSc in a Japanese population. In addition, the current study indicated that a candidate gene approach based on the results of GWAS of other diseases that display pathologic signaling pathways or mechanisms similar to those associated with the disease being examined is an effective approach to identifying novel susceptibility genes.

It will be interesting to perform GWAS of Japanese patients with SSc and analyze the similarities and differences in the detected associations not only between Japanese and Europeans but also between Japanese patients with SSc and Japanese patients with RA.

ACKNOWLEDGMENT

We thank the staff of the BioBank Japan Project for collecting DNA samples from control subjects.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Terao had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Terao, Ohmura, Kawaguchi, Nishimoto, Kawasaki, Takehara, Furukawa, Kochi, Ota, Ikari, Sato, Tohma, Yamada, Yamamoto, Kubo, Yamanaka, Kuwana, Tsuchiya, Matsuda, Mimori.

Acquisition of data. Terao, Ohmura, Kawaguchi, Nishimoto, Kawasaki, Takehara, Furukawa, Kochi, Ota, Ikari, Sato, Tohma, Yamada, Yamamoto, Kubo, Yamanaka, Kuwana, Tsuchiya, Matsuda, Mimori.

Analysis and interpretation of data. Terao, Ohmura.

REFERENCES

1. Chifflot H, Fautrel B, Sordet C, Chatelus E, Sibilia J. Incidence and prevalence of systemic sclerosis: a systematic literature review. Semin Arthritis Rheum 2008;37:223–35.
2. Kawut SM, Taichman DB, Archer-Chicko CL, Palevsky HI, Kimmel SE. Hemodynamics and survival in patients with pulmonary arterial hypertension related to systemic sclerosis. Chest 2003;123:344–50.
3. Ioannidis JP, Vlachoyiannopoulos PG, Haidich AB, Medsger TA Jr, Lucas M, Michet CJ, et al. Mortality in systemic sclerosis: an international meta-analysis of individual patient data. Am J Med 2005;118:2–10.
4. Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: shifting paradigms, emerging opportunities. Nat Rev Rheumatol 2012;8:42–54.
5. Romano E, Manetti M, Guiducci S, Ceccarelli C, Allanore Y, Matucci-Cerinic M. The genetics of systemic sclerosis: an update. Clin Exp Rheumatol 2011;29:S75–86.
6. Arnett FC, Cho M, Chatterjee S, Aguilar MB, Reveille JD, Mayes MD. Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. Arthritis Rheum 2001;44:1359–62.
7. Terao C, Ohmura K, Katayama M, Takahashi M, Kokubo M, Diop G, et al. Myelin basic protein as a novel genetic risk factor in rheumatoid arthritis: a genome-wide study combined with immunological analyses. PLoS One 2011;6:e20457.
8. Radstake TR, Gorlova O, Rueda B, Martin JE, Alizadeh BZ, Palomino-Morales R, et al. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. Nat Genet 2010;42:426–9.
9. Allanore Y, Saad M, Dieude P, Avouac J, Distler JH, Amouyel P, et al. Genome-wide scan identifies TNIP1, PSORS1C1, and RHOB as novel risk loci for systemic sclerosis. PLoS Genet 2011;7:e1002091.
10. Gorlova O, Martin JE, Rueda B, Koeleman BP, Ying J, Teruel M, et al. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. PLoS Genet 2011;7:e1002178.
11. Rueda B, Broen J, Simeon C, Hesselstrand R, Diaz B, Suarez H, et al. The STAT4 gene influences the genetic predisposition to systemic sclerosis phenotype. Hum Mol Genet 2009;18:2071–7.
12. Dieude P, Guedj M, Wipff J, Avouac J, Fajardy I, Diot E, et al. Association between the IRF5 rs2004640 functional polymorphism and systemic sclerosis: a new perspective for pulmonary fibrosis. Arthritis Rheum 2009;60:225–33.
13. Gourh P, Agarwal SK, Divecha D, Assassi S, Paz G, Arora-Singh RK, et al. Polymorphisms in TBX21 and STAT4 increase the risk of systemic sclerosis: evidence of possible gene–gene interaction and alterations in Th1/Th2 cytokines. Arthritis Rheum 2009;60:3794–806.
14. Dieude P, Guedj M, Wipff J, Ruiz B, Riemekasten G, Airo P, et al. NLRP1 influences the systemic sclerosis phenotype: a new clue for the contribution of innate immunity in systemic sclerosis-related fibrosing alveolitis pathogenesis. Ann Rheum Dis 2011;70:668–74.
15. Bossini-Castillo L, Broen JC, Simeon CP, Beretta L, Vonk MC, Ortego-Centeno N, et al. A replication study confirms the association of TNFSF4 (OX40L) polymorphisms with systemic sclerosis in a large European cohort. Ann Rheum Dis 2011;70:638–41.
16. Dieude P, Guedj M, Truchetet ME, Wipff J, Revillod L, Riemekasten G, et al. Association of the CD226 Ser^{307} variant with systemic sclerosis: evidence of a contribution of costimulation pathways in systemic sclerosis pathogenesis. Arthritis Rheum 2011;63:1097–105.
17. Gourh P, Agarwal SK, Martin E, Divecha D, Rueda B, Bunting H, et al. Association of the C8orf13-BLK region with systemic sclerosis in North-American and European populations. J Autoimmun 2010;34:155–62.
18. Dieude P, Guedj M, Wipff J, Ruiz B, Riemekasten G, Matucci-Cerinic M, et al. Association of the TNFAIP3 rs5029939 variant

with systemic sclerosis in the European Caucasian population. Ann Rheum Dis 2010;69:1958–64.
19. Tsuchiya N, Kawasaki A, Hasegawa M, Fujimoto M, Takehara K, Kawaguchi Y, et al. Association of STAT4 polymorphism with systemic sclerosis in a Japanese population. Ann Rheum Dis 2009;68:1375–6.
20. Ito I, Kawaguchi Y, Kawasaki A, Hasegawa M, Ohashi J, Hikami K, et al. Association of a functional polymorphism in the IRF5 region with systemic sclerosis in a Japanese population. Arthritis Rheum 2009;60:1845–50.
21. Ito I, Kawaguchi Y, Kawasaki A, Hasegawa M, Ohashi J, Kawamoto M, et al. Association of the FAM167A–BLK region with systemic sclerosis. Arthritis Rheum 2010;62:890–5.
22. Hasebe N, Kawasaki A, Ito I, Kawamoto M, Hasegawa M, Fujimoto M, et al. Association of UBE2L3 polymorphisms with diffuse cutaneous systemic sclerosis in a Japanese population. Ann Rheum Dis 2012;71:1259–60.
23. Zhou X, Lee JE, Arnett FC, Xiong M, Park MY, Yoo YK, et al. HLA–DPB1 and DPB2 are genetic loci for systemic sclerosis: a genome-wide association study in Koreans with replication in North Americans. Arthritis Rheum 2009;60:3807–14.
24. Suzuki A, Kochi Y, Okada Y, Yamamoto K. Insight from genome-wide association studies in rheumatoid arthritis and multiple sclerosis. FEBS Lett 2011;585:3627–32.
25. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis: a genomewide study. N Engl J Med 2007;357:1199–209.
26. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med 2007;357:977–86.
27. Plenge RM, Cotsapas C, Davies L, Price AL, de Bakker PI, Maller J, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet 2007;39:1477–82.
28. Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, et al. Rheumatoid arthritis association at 6q23. Nat Genet 2007;39: 1431–3.
29. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75:330–7.
30. Diaz-Gallo LM, Gourh P, Broen J, Simeon C, Fonollosa V, Ortego-Centeno N, et al. Analysis of the influence of PTPN22 gene polymorphisms in systemic sclerosis. Ann Rheum Dis 2011; 70:454–62.
31. Okada Y, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, et al. Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. Nat Genet 2012;44:511–6.
32. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
33. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988;15:202–5.
34. 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. Nature 2010;467: 1061–73.
35. Mimura Y, Ihn H, Jinnin M, Asano Y, Yamane K, Yazawa N, et al. Rheumatoid factor isotypes and anti-agalactosyl IgG antibodies in systemic sclerosis. Br J Dermatol 2004;151:803–8.
36. Santiago M, Baron M, Miyachi K, Fritzler MJ, Abu-Hakima M, Leclercq S, et al. A comparison of the frequency of antibodies to cyclic citrullinated peptides using a third generation anti-CCP assay (CCP3) in systemic sclerosis, primary biliary cirrhosis and rheumatoid arthritis. Clin Rheumatol 2008;27:77–83.
37. Yoshikawa F, Banno Y, Otani Y, Yamaguchi Y, Nagakura-Takagi Y, Morita N, et al. Phospholipase D family member 4, a transmembrane glycoprotein with no phospholipase D activity, expression in spleen and early postnatal microglia. PLoS One 2010;5: e13932.
38. Otani Y, Yamaguchi Y, Sato Y, Furuichi T, Ikenaka K, Kitani H, et al. PLD4 is involved in phagocytosis of microglia: expression and localization changes of PLD4 are correlated with activation state of microglia. PLoS One 2011;6:e27544.
39. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, Thorne N, et al. Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 2007;315: 848–53.
40. Cunninghame Graham DS, Morris DL, Bhangale TR, Criswell LA, Syvanen AC, Ronnblom L, et al. Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus. PLoS Genet 2011;7:e1002341.
41. Higgs BW, Liu Z, White B, Zhu W, White WI, Morehouse C, et al. Patients with systemic lupus erythematosus, myositis, rheumatoid arthritis and scleroderma share activation of a common type I interferon pathway. Ann Rheum Dis 2011;70:2029–36.
42. Whitfield ML, Finlay DR, Murray JI, Troyanskaya OG, Chi JT, Pergamenschikov A, et al. Systemic and cell type-specific gene expression patterns in scleroderma skin. Proc Natl Acad Sci U S A 2003;100:12319–24.
43. Lafyatis R, Kissin E, York M, Farina G, Viger K, Fritzler MJ, et al. B cell depletion with rituximab in patients with diffuse cutaneous systemic sclerosis. Arthritis Rheum 2009;60:578–83.
44. Smith V, Van Praet JT, Vandooren B, Van der Cruyssen B, Naeyaert JM, Decuman S, et al. Rituximab in diffuse cutaneous systemic sclerosis: an open-label clinical and histopathological study. Ann Rheum Dis 2010;69:193–7.
45. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, et al. IRF8 mutations and human dendritic-cell immunodeficiency. N Engl J Med 2011;365:127–38.

INTERNATIONAL JOURNAL OF UROLOGY

International Journal of Urology (2013) doi: 10.1111/iju.12074

Original Article

Prevalence of postmicturition urinary incontinence in Japanese men: Comparison with other types of incontinence

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Abbreviations & Acronyms
BMI = body mass index
ICIQ-SF = International Consultation Society Incontinence Questionnaire Short Form
MUI = mixed urinary incontinence
OR = odds ratio
PUI = postmicturition urinary incontinence
SUI = stress urinary incontinence
UI = urinary incontinence
UUI = urge urinary incontinence

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Received 25 September 2012; accepted 9 December 2012.

Objective: To investigate the prevalence and correlates of postmicturition urinary incontinence in Japanese men, and to compare with those of other types of urinary incontinence.

Methods: A total of 3224 male participants in a community-based survey were investigated. Three types of urinary incontinence were assessed; that is, postmicturition urinary incontinence, stress urinary incontinence and urge urinary incontinence. Age, body mass index, alcohol intake, cigarette smoking, and medical history of 18 diseases and conditions were the dependent variables for candidate correlates of the three types of incontinence.

Results: Unlike stress urinary incontinence and urge urinary incontinence, the prevalence of postmicturition urinary incontinence was constant throughout all generations (6.5% for the 30 s, 6.6% for the 40 s, 6.0% for the 50 s, 6.3% for the 60 s and 5.1% for the 70 s). The independent correlates for postmicturition urinary incontinence were asthma ($P < 0.001$; odds ratio 3.01), prostatic disease ($P < 0.001$; odds ratio 2.38), rhinosinusitis ($P = 0.001$; odds ratio 1.92), low back pain ($P = 0.003$; odds ratio 1.58), sleeplessness ($P = 0.013$; odds ratio 1.86), depression ($P = 0.024$; odds ratio 3.41) and body mass index ($P = 0.025$; odds ratio 0.73).

Conclusions: Postmicturition urinary incontinence has different characteristics from those of stress urinary incontinence and urge urinary incontinence. Unlike stress urinary incontinence and urge urinary incontinence, postmicturition urinary incontinence is not age-dependent. Several diseases related to an allergic status, such as asthma and rhinosinusitis, are correlates for postmicturition urinary incontinence.

Key words: epidemiology, male, urinary incontinence.

Introduction

UI affects substantial proportions of men. The estimated prevalence of UI increases with age, from 4.8% in men aged 19–44 years to 21.1% in men older than 65 years.[1] As most previous epidemiological studies assessing male UI focused on UUI, SUI and MUI, the prevalences and risk factors for these types of UI have been well investigated. These types of UI in men, especially UUI, result in considerable distress and deterioration of the quality of life.[2,3]

Postmicturition dribble is another type of UI, and is classified as a postmicturition symptom. This symptom has been defined by the International Continence Society in 2002 as involuntary loss of urine immediately after the individual has finished passing urine, usually after leaving the toilet in men,[4] and is distinct from terminal dribbling.[5] Several studies have found that the prevalence of postmicturition dribble is relatively high in men,[2,6–9] and the degree of distress caused by this symptom is substantial.[2] However, more

detailed information about this symptom, such as its age-stratified prevalence and correlates, is scarce.[8] In the present study, we investigated the prevalence and correlates of male postmicturition dribble using data from a Japanese cohort population, in comparison with those of other types of UI. We refer to postmicturition dribble as PUI hereafter, because we want to emphasize an aspect of urinary incontinence with this symptom for comparisons with other types of UI.

Methods

The Nagahama cohort project is a prospective study composed of a questionnaire survey, anthropometric measures, physiological measures, biochemical measures from blood samples and genomic information.[10] The baseline data for the study population were obtained from August 2008 to November 2010. All the protocols and informed consent procedures were approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee, the ad hoc Review Board of the Nagahama Cohort Project, and the Nagahama Municipal Review Board of Personal Information Protection.

Although the questionnaire was designed to be self-reporting, the interviewers confirmed the appropriate answer by interview if no answer was written to minimize the lack of data. Self-reported information on the medical history, major comorbidities, current medication use, lifestyle and psychosocial factors were also collected. The three types of UI; that is, SUI, UUI and PUI, were assessed using the ICIQ-SF, the Japanese version of which has been validated for use.[11] When assessing the prevalence of UI, we examined all severities of UI; that is, "about once a week or less often" or more in frequency and "a small amount" or more in volume. When assessing the severity of UI, we classified it into the following three levels: mild, once or less per week; moderate, two or three times per week; severe, once or more per day.

The independent variables used in the analyses for correlates of the three types of incontinence were age; BMI; alcohol intake; cigarette smoking; and 18 medical history diseases and conditions, comprising low back pain, hypertension, rhinosinusitis, dyslipidemia, prostatic disease, diabetes mellitus, gout, sleeplessness, hepatic disease, coronary artery disease, anemia, malignant disease, atopic dermatitis, asthma, depression, reflux esophagitis, renal disease and stroke. Several diseases or conditions included in the questionnaire were omitted from the analyses because of their low prevalence or relation to injury.

Statistics

Statistical analyses were carried out using a logistic regression model. Univariate analyses were first carried out to confirm the basic relationships between each independent variable and the three types of UI. If the univariate analysis produced a *P*-value of less than 0.25, the variable was applied to a multivariate analysis. All *P*-values were two-sided, and values of $P < 0.05$ were considered significant. SPSS version 13.0 (SPSS, Chicago, IL, USA) was used for all calculations.

Table 1 Characteristics of the participants

Age (years)	
30–39	673 (20.9%)
40–49	381 (11.8%)
50–59	481 (14.9%)
60–69	1201 (37.3%)
70–	488 (15.1%)
BMI (kg/m^2)	
<18.5	108 (3.3%)
18.5–24.9	2243 (69.6%)
25.0–29.9	776 (24.1%)
30≤	97 (3.0%)
Alcohol (yes)	2617 (81.2%)
Smoking (yes)	991 (30.7%)
Medical conditions	
Low back pain (yes)	1743 (54.1%)
Hypertension (yes)	791 (24.5%)
Rhinosinusitis (yes)	385 (11.9%)
Dyslipidemia (yes)	366 (11.3%)
Prostatic disease (yes)	299 (9.3%)
Diabetes (yes)	295 (9.2%)
Gout (yes)	237 (7.4%)
Sleeplessness (yes)	211 (6.5%)
Hepatic disease (yes)	184 (5.7%)
Coronary artery disease (yes)	176 (5.5%)
Anemia (yes)	159 (4.9%)
Malignant disease (yes)	144 (4.5%)
Atopic dermatitis (yes)	136 (4.2%)
Asthma (yes)	127 (3.9%)
Depression (yes)	115 (3.6%)
Reflux esophagitis (yes)	104 (3.2%)
Renal disease (yes)	87 (2.7%)
Stroke (yes)	38 (1.2%)

Results

A total of 3228 male residents participated in the Nagahama project. Of these, four residents were excluded from the study because of insufficient data. The data for the remaining 3224 (99.9%) residents were evaluated in the study. The characteristics and distributions of the participants are shown in Table 1.

Overall, 441 residents (13.7%) had some type of UI. Of these, 334 (10.4%), 46 (1.4%) and 61 (1.9%) residents had mild, moderate and severe UI, respectively. The prevalences of mild UI and moderate to severe UI increased with aging

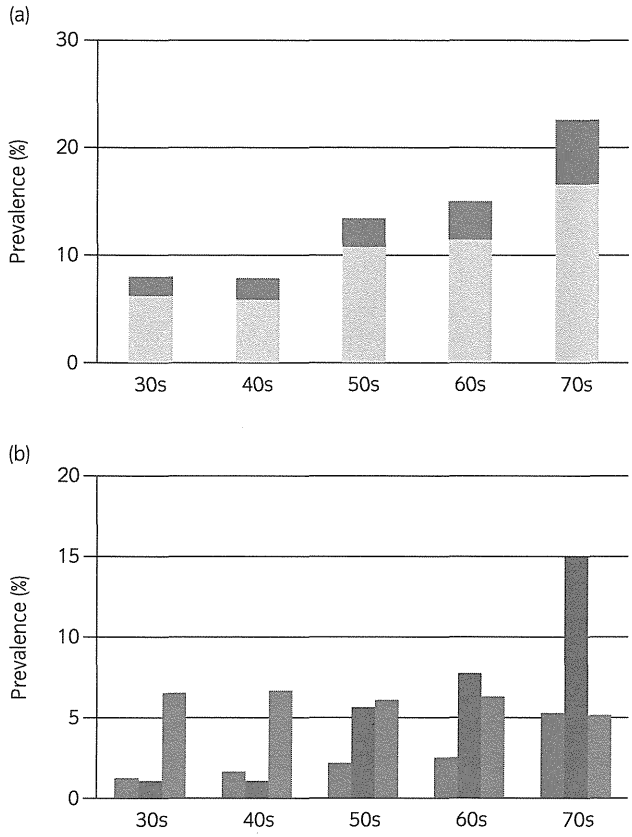

Fig. 1 (a) Prevalences of mild UI and moderate to severe UI stratified by age. (b) Prevalences of the three types of UI stratified by age. ■, moderate to severe UI; ■, mild UI; ■, SUI; ■, UUI; ■, PUI.

(Fig. 1a). Regarding the types of UI, 79 residents (2.5%) had SUI, 204 (6.3%) had UUI and 199 (6.2%) had PUI. A total of 15 residents (0.5%) had MUI. As shown in Figure 1b, the prevalences of SUI and UUI increased with aging, whereas the prevalence of PUI did not change.

The results of the analyses for correlates of each type of UI are shown in Tables 2 and 3. The univariate analyses showed that prostatic disease, age, malignant disease, anemia and hypertension were associated with SUI (Table 2). Among these variables, prostatic disease (OR 2.14), age (OR 1.29), anemia (OR 2.27) and malignant disease (OR 2.22) were independent correlates for SUI in the multivariate analyses (Table 3). Although both prostatic disease and malignant disease were independently correlated with SUI, the number of participants with prostate cancer was 22, which accounted for 10% of all participants with prostatic disease and 15% of all participants with malignant disease. Age, low back pain, diabetes, prostatic disease, hypertension, coronary artery disease, renal disease, malignant disease, sleeplessness, hepatic disease, dyslipidemia, BMI and alcohol intake were associated with UUI in the univariate analyses (Table 2). Among these variables, diabetes (OR 1.98), age (OR 1.92), low back pain (OR 1.74), alcohol intake (OR 1.84), renal disease (OR: 1.91) and prostatic disease (OR 1.48) remained statistically significant in the multivariate analyses (Table 3).

Unlike SUI and UUI, age was not associated with PUI. In contrast, asthma, prostatic disease, rhinosinusitis, sleeplessness, reflux esophagitis, anemia and BMI were associated with PUI in the univariate analyses (Table 2). Among these variables, asthma (OR 3.01), prostatic disease (OR 2.38), rhinosinusitis (OR 1.92), low back pain (OR 1.58) and BMI (OR 0.73) remained statistically significant in the multivariate analyses. Depression was another independent correlate for PUI (OR 3.41). Reflux esophagitis was marginally associated with PUI ($P = 0.09$) (Table 3). The lack of association between age and PUI was also observed after adjustment for the other independent correlates.

Discussion

The development of the three types of UI in men is deemed to be caused by different mechanisms. UUI, which is a component symptom of overactive bladder syndrome, is usually attributed to detrusor overactivity. SUI is induced by dysfunction of the urethral sphincter, which typically results from iatrogenic causes, such as prostatectomy. Meanwhile, the mechanism for the development of PUI is not well recognized. An earlier study by Stephenson *et al.* showed that most patients with PUI had normal urodynamic bladder function.[5] That study also found that half of the patients could not occlude their urethra voluntarily or "milk back" residual urine in the urethra into the bladder, suggesting contraction failure of the bulbocavernosus muscle. However, another study found that patients with PUI had normal reflex and activity of the bulbocavernosus muscle, as assessed by electromyography.[12] Despite the lack of an obvious mechanism, residual urine in the bulbar urethra has been attributed to PUI.[5]

The different mechanisms naturally suggest different prevalences and correlates of the three types of UI, and this was confirmed in the present study. Among the independent correlates of UUI, age, history of diabetes and low back pain had especially strong associations with UUI. Aging and diabetes have frequently been reported as risk factors for UI. Diabetes can induce various types of bladder dysfunctions, including detrusor overactivity,[13] and therefore the association of diabetes with UUI is reasonable. Low back pain is a novel correlate of UUI. Although this symptom can arise from various problems, age-related degenerative processes in the intervertebral disks and facet joints, spinal stenosis, and disk herniation are considered to be common causes.[14] These structural deformations of the spine might induce compression of the spinal cord or spinal roots, and potentially result in neurogenic bladder dysfunction, such as detrusor overactivity. Conversely, one longitudinal study

Table 2 Univariate analyses for correlates of SUI, UUI and PUI

	SUI		UUI		PUI	
	P-value	OR	*P*-value	OR	*P*-value	OR
Age	<0.001	1.44	<0.001	2.03	0.43	–
BMI	0.40	–	0.034	0.75	0.019	0.72
Alcohol	0.51	–	0.042	0.65	0.36	–
Smoking	0.40	–	0.66	–	0.58	–
Low back pain	0.39	–	<0.001	1.82	<0.001	1.74
Hypertension	0.044	1.62	<0.001	1.88	0.57	–
Rhinosinusitis	0.88	–	0.090	–	<0.001	2.16
Dyslipidemia	0.99	–	0.014	1.62	0.93	–
Prostatic disease	<0.001	3.25	<0.001	2.80	<0.001	2.80
Diabetes	0.48	–	<0.001	2.95	0.77	–
Gout	0.168	–	0.58	–	0.65	–
Sleeplessness	0.40	–	0.005	1.93	0.001	2.09
Hepatic disease	0.80	–	0.010	1.89	0.91	–
Coronary artery disease	0.40	–	0.001		0.050	1.68
Anemia	0.002	2.91	0.52	–	0.017	1.90
Malignant disease	<0.001	3.70	0.002	2.24	0.26	–
Atopic dermatitis	0.85	–	0.56	–	0.222	–
Asthma	0.28	–	0.47	–	<0.001	3.05
Depression	0.29	–	0.78	–	0.229	–
Reflux esophagitis	0.35	–	0.32	–	0.008	2.25
Renal disease	0.44	–	0.001	2.71	0.106	–
Stroke	0.27	–	0.79	–	0.82	–

showed that incontinence increased the risk of future back pain.[15] In any case, low back pain has a close relationship with UUI. PUI, as well as UUI, had an independent association with low back pain. The common symptom of low back pain would be better noted as an associated factor for various lower urinary tract symptoms including UUI and PUI. Similar to the case for UUI, aging was also a strong correlate of SUI. The other independent correlates of SUI were history of prostatic disease, malignant disease and anemia. Some previous studies have reported that men with prostatic disease have higher rates of UI.[16,17] Although it is well known that SUI is one of the most important complications after prostatectomy for prostatic diseases, especially prostate cancer, the present study population had a low prevalence of prostate cancer. Therefore, the association between SUI and prostatic disease and/or malignant disease in the present study would not be mainly attributable to radical prostatectomy.

The most striking observation in the present study was the lack of correlation between aging and PUI. The prevalence of PUI was found to remain constant at 5–6% throughout the generations, and the severity and frequency of this symptom were also not age dependent (data not shown). These observations suggest that this symptom does not arise through mechanical or functional dysfunction induced by aging. Furthermore, the independent correlates of PUI included uncommon parameters, such as asthma and rhinosinusitis; along with relatively common parameters, such as prostatic disease, sleeplessness and depression. There was also a marginal association between reflux esophagitis and PUI. Although some recent studies have suggested a relationship between overactive bladder and asthma,[18,19] asthma had no association with UUI, but was associated with PUI in the present study. It is recognized that asthma and rhinitis/rhinosinusitis have a strong correlation with each other.[20] The most accepted explanation for the interaction between the upper and lower airways is increased oral breathing and systemic response. In other words, impaired filtering in the nose of patients with rhinitis leads to mouth breathing, which can result in increased exposure of the lower airways to allergens. It is also known that asthmatic patients have a much greater risk of gastro-esophageal reflux disease-related symptoms and vice versa.[20] Gastro-esophageal reflux disease, including reflux esophagitis, could worsen asthma either by direct effects on airway responsiveness or through aspiration-induced inflammation, and the bronchoconstriction observed in asthma, as well as asthma medications, could induce gastro-esophageal reflux. Although these three diseases, asthma, rhinosinusitis and reflux esophagitis, have associations with one another and with allergy, the present

Table 3 Multivariate analyses for correlates of SUI, UUI and PUI

	SUI			UUI			PUI		
	P-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI
Age	0.013	1.29	1.06–1.57	<0.001	1.92	1.64–2.24	–		
BMI	–			0.10			0.025	0.73	0.55–0.96
Alcohol	–			0.005	1.84	1.20–2.82	–		
Smoking	–			–			–		
Low back pain	–			<0.001	1.74	1.29–2.34	0.003	1.58	1.17–2.12
Hypertension	0.40			0.59			–		
Rhinosinusitis	–			0.12			0.001	1.92	1.33–2.77
Dyslipidemia	–			0.36			–		
Prostatic disease	0.009	2.14	1.21–3.77	0.045	1.48	1.01–2.18	<0.001	2.38	1.63–3.48
Diabetes	–			<0.001	1.98	1.36–2.90	–		
Gout	0.28			–			–		
Sleeplessness	–			0.33			0.013	1.86	1.14–3.04
Hepatic disease	–			0.24			–		
Coronary artery disease	–			0.42			0.33		
Anemia	0.022	2.27	1.13–4.57	–			0.26		
Malignant disease	0.025	2.22	1.10–4.45	0.53			–		
Atopic dermatitis	–			–			0.10		
Asthma	–			–			<0.001	3.01	1.79–5.06
Depression	–			–			0.024	3.41	1.17–9.90
Reflux esophagitis	–			–			0.09		
Renal disease	–			0.045	1.91	1.01–3.60	0.36		
Stroke	–			–			–		

study showed that each of these diseases was independently associated with PUI. As the mechanism for the development of PUI is completely unknown, as aforementioned, we cannot even speculate about the reasons why these diseases have relationships with PUI. Further investigations in this area are warranted in the future.

There have been a few epidemiological studies investigating the prevalence of PUI.[6,8,9] The previously reported results for the prevalence of PUI were quite variable ranging from 5.5 to 38.3%. Including the present study, the highly variable percentages might result from different questioning methods, interviews or self-reporting, and different races of the study populations. However, the most likely reason for the difference would be subtle differences in the formulation of questions indicating PUI. We used the ICIQ-SF, whereas others used a questionnaire based on the International Continence Society definition. Every question used in studies on the prevalence of PUI is validated for use, and the results obtained are significant. However, it is noteworthy that subtle differences in the formulation of questions can influence the outcomes.

The present study had several limitations. One is the ambiguity in the names of several diseases/conditions. For example, prostatic disease includes benign prostatic hyperplasia, prostate cancer and prostatitis, and we cannot distinguish concrete disease entities. Low back pain is also an ambiguous name for a symptom that can result from various diseases as described earlier. However, in actuality, many of the participants, who are amateurs in the medical field, did not know the exact disease causing their symptom of low back pain. Another limitation was the lack of data on bladder or bowel diseases, which can affect the function of the lower urinary tract. Despite these limitations, we believe that our observations provide some clues for the future solution of the mechanism of PUI.

Conflict of interest

None declared.

References

1 Shamliyan TA, Wyman JF, Ping R, Wilt TJ, Kane RL. Male urinary incontinence: prevalence, risk factors, and preventive interventions. *Rev. Urol.* 2009; **11**: 145–65.
2 Engstrom G, Engstrom MLW, Henningsohn L, Loof L, Leppert J. Prevalence of distress and symptoms severity from the lower urinary tract in men: a population-based study with the DAN-PSS questionnaire. *Fam. Pract.* 2003; **21**: 617–22.

3 Coyne KS, Kvasz M, Ireland AM, Milsom I, Kopp ZS, Chapple CR. Urinary incontinence and its relationship to mental health and health-related quality of life in men and women in Sweden, the United Kingdom, and the United States. *Eur. Urol.* 2012; **61**: 88–95.
4 Abrams P, Cardozo L, Fall M *et al.* The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. *Neurourol. Urodyn.* 2002; **21**: 167–78.
5 Stephenson TP, Farrar DJ. Urodynamic study of 15 patients with postmicturition dribble. *Urology* 1977; **9**: 404–6.
6 Malmsten UG, Molander U, Peeker R, Irwin DE, Milsom I. Urinary incontinence, overactive bladder, and other lower urinary tract symptoms: a longitudinal population-based survey in men aged 45–103 years. *Eur. Urol.* 2010; **58**: 149–56.
7 Hrisanfow E, Hagglund D. The prevalence of urinary incontinence among women and men with chronic obstructive pulmonary disease in Sweden. *J. Clin. Nurs.* 2011; **20**: 1895–905.
8 Irwin DE, Milsom I, Hunskaar S *et al.* Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: results of the EPIC study. *Eur. Urol.* 2006; **50**: 1306–15.
9 Coyne KS, Sexton CC, Thompson CL *et al.* The prevalence of lower urinary tract symptoms (LUTS) in the USA, the UK and Sweden: results from the Epidemiology of LUTS (EpiLUTS) study. *BJU Int.* 2009; **104**: 352–60.
10 Nakayama T. Implementation of a genomic epidemiology and public health activities in a community: establishing a "rule" for the protection and utilization of individual information. *Jpn. J. Genet. Counsel.* 2010; **31**: 117–21.
11 Gotoh M, Donovan J, Corcos J *et al.* Scored ICIQ-SF (International Consultation on Incontinence Questionnaire-Short Form) for symptoms and QOL assessment in patients with urinary incontinence. *Nippon Hainyokino Gakkaishi* 2001; **12**: 227–31 (in Japanese).
12 Paquin JM, D'Amour M, Perreault JP, Faucher R, Mauffette F. Electromyographic study of bulbocavernosus muscle in 15 patients with postmicturition dribbling. *Urology* 1985; **26**: 603–5.
13 Daneshgari F, Liu G, Birder L, Mitchell ATH, Chacko S. Diabetic bladder dysfunction: current translational knowledge. *J. Urol.* 2009; **182**: S18–26.
14 Deyo RA, Weinstein JN. Low back pain. *N. Engl. J. Med.* 2001; **344**: 363–70.
15 Smith MD, Russell A, Hodges PW. Do incontinence, breathing difficulties, and gastrointestinal symptoms increase the risk of future back pain? *J. Pain* 2009; **10**: 876–86.
16 Van Oyen H, van Oyen P. Urinary incontinence in Belgium: prevalence, correlates and psychosocial consequences. *Acta Clin. Belg.* 2002; **57**: 207–18.
17 Markland AD, Goode PS, Burgio KL *et al.* Correlates of urinary, fecal, and dual incontinence in older African-American and white men and women. *J. Am. Geriatr. Soc.* 2008; **56**: 285–90.
18 Coyne KS, Sexton CC, Irwin DE, Kopp ZS, Kelleher CJ, Milsom I. The impact of overactive bladder, incontinence and other lower urinary tract symptoms on quality of life, work productivity, sexuality and emotional well-being in men and women: results from the EPIC study. *BJU Int.* 2008; **101**: 1388–95.
19 Correia S, Dinis P, Rolo F, Lunet N. Prevalence, treatment and known risk factors of urinary incontinence and overactive bladder in the non-institutionalized Portuguese population. *Int. Urogynecol. J.* 2009; **20**: 1481–9.
20 Boulet LP, Boulay ME. Asthma-related comorbidities. *Expert Rev. Respir. Med.* 2011; **5**: 377–93.

Appendix I

The Nagahama Cohort Research Group

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PLOS ONE

Three Groups in the 28 Joints for Rheumatoid Arthritis Synovitis – Analysis Using More than 17,000 Assessments in the KURAMA Database

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Abstract

Rheumatoid arthritis (RA) is a joint-destructive autoimmune disease. Three composite indices evaluating the same 28 joints are commonly used for the evaluation of RA activity. However, the relationship between, and the frequency of, the joint involvements are still not fully understood. Here, we obtained and analyzed 17,311 assessments for 28 joints in 1,314 patients with RA from 2005 to 2011 from electronic clinical chart templates stored in the KURAMA (Kyoto University Rheumatoid Arthritis Management Alliance) database. Affected rates for swelling and tenderness were assessed for each of the 28 joints and compared between two different sets of RA patients. Correlations of joint symptoms were analyzed for swellings and tenderness using kappa coefficient and eigen vectors by principal component analysis. As a result, we found that joint affected rates greatly varied from joint to joint both for tenderness and swelling for the two sets. Right wrist joint is the most affected joint of the 28 joints. Tenderness and swellings are well correlated in the same joints except for the shoulder joints. Patients with RA tended to demonstrate right-dominant joint involvement and joint destruction. We also found that RA synovitis could be classified into three categories of joints in the correlation analyses: large joints with wrist joints, PIP joints, and MCP joints. Clustering analysis based on distribution of synovitis revealed that patients with RA could be classified into six subgroups. We confirmed the symmetric joint involvement in RA. Our results suggested that RA synovitis can be classified into subgroups and that several different mechanisms may underlie the pathophysiology in RA synovitis.

Citation: Terao C, Hashimoto M, Yamamoto K, Murakami K, Ohmura K, et al. (2013) Three Groups in the 28 Joints for Rheumatoid Arthritis Synovitis – Analysis Using More than 17,000 Assessments in the KURAMA Database. PLoS ONE 8(3): e59341. doi:10.1371/journal.pone.0059341

Editor: Bernhard Kaltenboeck, Auburn University, United States of America

Received October 21, 2012; **Accepted** February 12, 2013; **Published** March 12, 2013

Funding: This study was supported by research grants from Mitsubishi Tanabe Pharma Corporation (http://www.mt-pharma.co.jp/e/), Eisai Co., Ltd. (http://www.eisai.co.jp/index.html), Abbott Japan Co., Ltd. (http://www.abbott.co.jp/), Chugai Pharmaceutical Co., Ltd. (http://www.chugai-pharm.co.jp/hc/ss/english/index.html), Pfizer Japan Inc. (http://www.pfizer.co.jp/pfizer/english/company/), and Bristol-Myyers K.K. (http://www.bms.co.jp/). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding was received for this study.

Competing Interests: The KURAMA database was supported by funding from Mitsubishi Tanabe Pharma Corporation, Eisai Co., Ltd., Abbott Japan Co., Ltd., Chugai Pharmaceutical Co., Ltd., Pfizer Japan Inc. and Bristol-Myyers. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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Introduction

Rheumatoid arthritis (RA) is the most frequent inflammatory arthritis worldwide affecting 0.5 to 1% of the population [1]. As RA is a bone-destructive disease and functional impairment caused by joint damage is well correlated with swelling and tenderness of joints [2–3], the evaluation of joints in patients with RA is very important to assess disease activity and predict the risk of future joint deformity. ACR core set [4] and DAS (disease activity score) [5–6] were developed for evaluation of disease activity in RA. Recently, the three composite indices, namely, DAS28 [5], simplified disease activity index (SDAI) [7] and clinical disease activity index (CDAI) [8] are frequently used for disease activity evaluation among rheumatologists. All of the three indices are shown to be well correlated with future joint destruction [7,9]. These three methods include the same 28 joints for evaluation of disease activity, namely, bilateral wrist, 1st to 5th metacarpal (MCP) joints and proximal interphalangeal (PIP) joints, elbow, shoulder, and knee joints. Though RA is known to show symmetric joint symptoms [10], the frequency of bilateral joint symptoms and the correlations between each joint symptom are not fully analyzed by using large numbers of joint assessments. There are several reports of successful prediction of joint damage using a reduced number of joints for evaluation by ultrasonogra-

phy [11–12]. These reports raise the possibility that some of the 28 joints are less frequently involved, and are less informative for disease activity. Analyses for characterization of joint symptoms would uncover correlations of unexpected joint symptoms and distribution of synovitis in RA.

Here, we analyzed the distribution of affected joints in the 28 joints in patients with RA using more than 17,000 joint assessments from 1,314 patients with RA and showed that synovitis in RA patients can be classified into three groups. We also showed that affected rates of the 28 joints greatly vary in RA patients, and that RA patients could be classified into subgroups based on the distribution of joint synovitis.

Results

Frequency order of joints involvement

We recruited 17,311 assessments for the 28 joints in 1,314 patients with RA from 2005 to 2011. A summary of the registered patients is listed in Table 1. The distribution of the number of patients with RA in each year and the number of joint assessments for each patient are shown in Figure S1. We analyzed how often each of the 28 joints was tender or swollen in patients with RA in 2011. From the analysis of 735 patients, we found that the frequency of joint swelling and tenderness in the 28 joints is widely different from joint to joint (Figure 1 and Table S1). The wrist joints were the most frequently affected joints for swelling and tenderness. The frequency of the right wrist joint being affected was more than four times as high as the least frequently affected joint. Many of the joints showed right-dominant tenderness (eleven of fourteen joints, $p = 0.057$, binomial test), indicating mostly right-handedness. We found strong correlations for the affected rates of each joint between swellings and tenderness except for shoulder joints (Spearman's rank-sum coefficient, rho = 0.70 and $p = 3.8\times10^{-5}$, Figure 1, Table S1). Shoulder joints showed much higher frequencies of tenderness than those of swellings.

Next, we tried to replicate the order of affected frequencies of the 28 joints and the correlation between tenderness and swellings in different RA patients. We obtained 579 patients whose joints data were not available for 2011, indicating we analyzed independent RA patients. We found that the order of the affected joint frequencies were well correlated for both swelling and tenderness among different sets of RA patients (Spearman's rank-sum coefficient, rho:0.815 and 0.904, $p = 1.3\times10^{-7}$ and $p = 4.6\times10^{-11}$ for swelling and tenderness, respectively, Figure S2). We also confirmed that rates of tenderness were well correlated with those of swellings in the 28 joints in the 579 patients (rho:0.604). These results indicate that some of the 28 joints are more likely to develop arthritis than the others in RA patients. The swelling and tenderness correlate with each other except for shoulder joints.

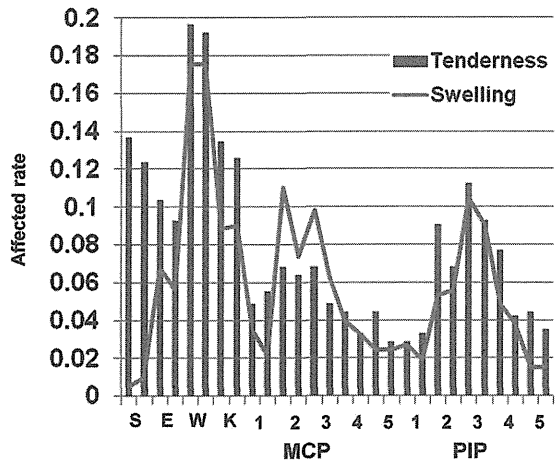

Figure 1. Affected rate of joint symptoms. Affected rate of joint symptoms. Each joint is arranged in the order of right and left. S:shoulder, E:elbow, W:wrist, K:knee.
doi:10.1371/journal.pone.0059341.g001

Table 1. Summary of the KURAMA database.

	The KURAMA database
RA patients	1314
Age (mean±SD)	60.2±15.1
female ratio	81.70%
disease duration (years)	12.2±9.8
Stage*	2.75±1.17
Class*	1.87±0.69

*Stage and Class represent Steinbrocker's stage and class, respectively.
SD: standard deviation.
doi:10.1371/journal.pone.0059341.t001

Whether the right-dominant involvement of joints in patients with RA is associated with joint destruction was analyzed. Joint destruction in the hand was evaluated for 246 patients with RA by modified Sharp score [13]. The six elements of the scores were separately analyzed, namely erosion of PIP, MCP, and wrist joints (we defined as joints other than MCP and PIP in hand) and narrowing of PIP, MCP, and wrist joints. We found that five out of six elements showed right-dominant destruction. In particular, narrowing and erosion of MCP joints showed a statistically significant right-dominance in binomial test ($p <= 0.0050$, Table S2).

Three groups of 28 joints in RA synovitis

Next we analyzed correlations of joint symptoms between the 28 joints. We randomly picked up one assessment from each of the 1,314 patients to maximize the power. When the correlation of tenderness of the 28 joints was analyzed with kappa coefficient, we confirmed that each joint showed a symmetric involvement (Figure 2A). The results also showed that the tenderness of large joints and wrist joints are not correlated with the tenderness of PIP and MCP joints. We found that the tenderness of MCP joints was especially well correlated with each other and that PIP joints tenderness was well correlated with each other. The correlation of swelling in the 28 joints showed the same tendency as that of tenderness, namely, symmetric joint involvement, correlations between large joints and wrist joints, and no strong correlations between wrist joints and other small joints (Figure 2B).

Next we used eigen vectors of principal component analysis to assess the correlations of the 28 joints involvement. When we analyzed correlations of tenderness, eigen vectors revealed that PIP and MCP joints can be clearly distinguished from large joints and wrist joints (Figure 3A). PIP joints and MCP joints turned out to make independent groups after excluding large joints and wrist joints (Figure 3B). These three groups of affected joints were found both for tenderness and swelling (Figure 3C and 3D). We confirmed these three correlation groups in four independent resampling analyses by randomly picking up one assessment from each of the 1,314 patients four times (data not shown). The three groups were observed in the two independent sets of RA patients which were used in the analysis of joints involvement frequency

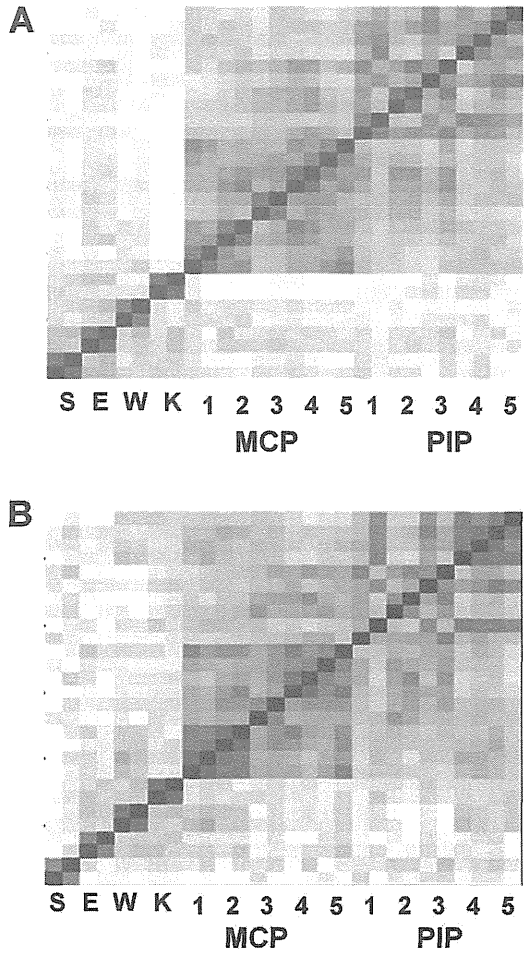

Figure 2. Correlations between the 28 joint symptoms. Brightness of the red color corresponds to the strength of correlations between joint tenderness (A) or swellings (B), using the Kappa coefficient. Each joint is arranged in the order of right and left. The joint order in the y axis is the same as the x axis. The result is a representative of five analyses based on resampled assessments. S:shoulder, E:elbow, W:wrist, K:knee.
doi:10.1371/journal.pone.0059341.g002

(Figure S3). In addition, no significant difference was observed in the relationship of the three groups of joint involvement when we divided the 1,314 patients into two groups according to the patients' caring physicians (Figure S4). We confirmed the three groups by resampling four times for each analysis (data not shown). These results indicate that these three groups were not due to specific patients, examiners, or time of evaluation.

Taken together, the correlation analyses using kappa coefficient and eigen vectors in principal component analysis indicated that there are three correlated groups of joints in RA synovitis, namely, large joints with wrist joints (which we express as "large and wrist joints"), PIP joints, and MCP joints.

Subgroups of patients with RA

We performed a clustering analysis of 5,383 evaluations of 28 joints from 1,314 patients with RA. Six subgroups of evaluations of 28 joints were observed (Figure 4). Each of the subgroups was characterized by 1) no synovitis (34.6%), 2) mild activity with dominant involvement of large and wrist joints (17.4%), 3) dominant involvement of MCP joints (18.3%), 4) dominant involvement of PIP joints (9.3%), 5) active synovitis (4.1%), and 6) moderate activity with dominant involvement of large and wrist joints (16.4%) (Table S3). Whether patients with RA are classified into the same subgroups was analyzed. There were 998 patients with four or five evaluations, and of these, 734 were categorized into the regular groups across different evaluations, indicating that the patterns of synovitis in the same patients were stable. Analysis of joint destruction in each subgroup revealed that the sixth subgroup demonstrated dominant destruction of large and wrist joints compared with MCP and PIP joints ($p <= 2.8 \times 10^{-5}$, Figure S5 and Figure S6).

Discussion

Since RA is a joint destructive autoimmune arthritis and joint damage occurs rapidly in the early stages of the disease course [14], the development of a quantitative scale which assesses disease activity and predicts joint damage is very important. After DAS and ACR core sets were introduced, DAS28, SDAI, and CDAI were developed to evaluate disease activity and easily calculate the disease activity score in patients with RA. All three indices were shown to be well correlated with future joint destruction and they share the same 28 joints for evaluation. Joint symptoms especially joint swelling is known to correlate with future joint damage [3]. While these indices were developed for use in clinical trials such as responsiveness to treatment, they are used by rheumatologists in daily clinical practice and they are reported to coincide very well among different examiners [9]. Characterizing the relative affected frequency of each joint and analysis of correlation between joint symptoms are important to analyze the basic mechanisms of synovitis and to efficiently select the joints to predict future joint destruction. However, there is no detailed analysis to address the correlations between the 28-joint symptoms.

In the current study, we characterized the 28-joint symptoms using large numbers of joint assessments. While we reported the affected rates of each joint in the 28 joints for tenderness and swelling of RA patients registered in the KURAMA database in 2011 as a representative (Table S1), these rates should not be generalized considering large effects of treatment especially biologics agents on joint symptoms. Thus, we focused on relative frequencies of joint involvement for the 28 joints. The affected frequency pattern was compared between the two sets of RA patients, and there were no apparent differences between the two sets for both tenderness and swelling. We also showed that joint symptoms in RA could be classified into three groups both for tenderness and swelling. Our analysis also demonstrated that patients with RA can be regularly classified into six subgroups based on patterns of joint symptoms. These results suggest that regular RA joint involvement pattern, including relative frequency and groups of joints, is largely maintained in RA patients. In addition, we confirmed that these patterns of joint involvement were not attributed to evaluators and fractions of RA patients.

It is interesting that the affected frequencies greatly varied from joint to joint, and the rate of the most highly affected joint was more than four times as high as the least-affected joint. The affected frequencies indicated that wrist joints were the most frequently affected. It should be noted that surface area may have influenced the sensitivity of detecting synovitis in physical exams when different joints were compared. The relatively high frequency of tenderness and swelling in large and wrist joints compared with MCP and PIP joints can be explained by this difference in surface area. However, surface area cannot fully explain the highest frequency of wrist involvement and different frequencies within the MCP or PIP joints. A dominant involve-

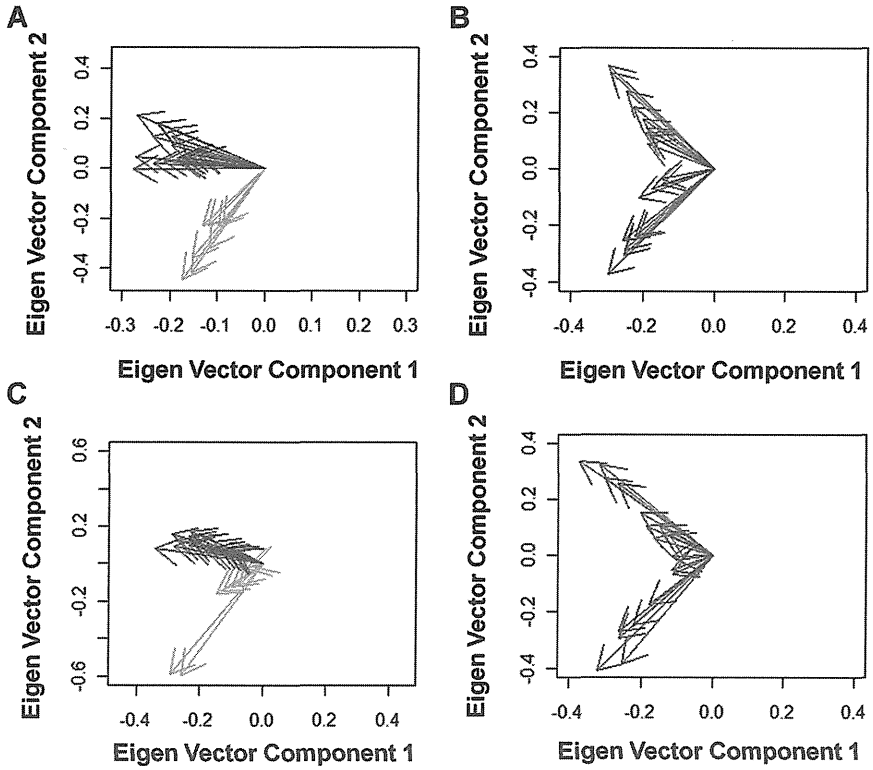

Figure 3. Relationship of the 28-joint involvement. The 1st and 2nd components of eigen vectors of the joint symptoms are plotted, using principal component analysis of the 28 joint involvement for tenderness (A) and swelling (C) or using that of the 20 joint involvement other than large and wrist joints for tenderness (B) and swelling (D). The results are representatives of five analyses based on resampled assessments. Green: large and wrist joints. Red: MCP joints. Blue: PIP joints.
doi:10.1371/journal.pone.0059341.g003

ment of right joints seemed to indicate a majority of the study population being right-handed in spite of the small difference of affected rates between bilateral joints. We also demonstrated that the right dominant involvement was also true for joint destruction. We could not compare the joint involvement and joint destruction between right-handed patients and left-handed patients due to a lack of information regarding handedness of patients.

Correlation analysis confirmed the well-known symmetric joint involvement in patients with RA. Strong correlations of tenderness and swelling in the same joints except for shoulder joints may indicate low sensitivity of shoulder swelling in the physical exams and common mechanisms of swelling and tenderness. It is striking that joint symptoms can be classified into three groups based on correlation analysis and principal component analysis. The association observed between the symptoms in the wrist joints and the large joints is worth noting, since wrist joints are regarded as small joints according to ACR/EULAR criteria set in 2010. As wrist joints are much closer to other small joints than large joints, the relationship between wrist joints and large joints cannot be explained by the distance of joints. The distance of joints cannot explain the two different groups of MCP and PIP joints either. While symptoms of large and wrist joints are not related with those of MCP and PIP joints, they were not very strongly correlated with each other, compared with correlations among PIP joints or MCP joints. This may indicate that there are no common strong factors which predispose large and wrist joints to swelling and tenderness in patients with RA.

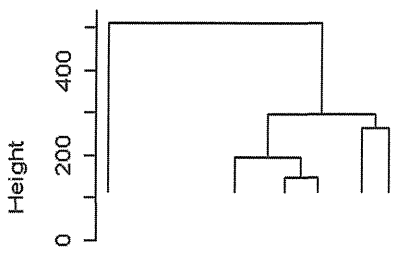

Figure 4. Six subgroups of evaluations of the 28 joints in RA. Results of clustering analysis with Ward method using randomly obtained 5,383 evaluations of the 28 joints in 1,314 patients were plotted.
doi:10.1371/journal.pone.0059341.g004

We also showed that patients with RA can be divided into six subgroups based on these three groups of joint involvement. More than 70% of patients are classified into regular subgroups, indicating that the pattern of synovitis in a patient with RA is stable. When patients who were regularly classified into the first subgroup of patients characterized by no synovitis were removed, more than 60% of patients were still classified into regular subgroups (data not shown), indicating that the stable patterns were observed regardless of activity of RA. As joint destruction was influenced by disease duration, disease activity, and treatment, we analyzed the relative distribution of joint destruction between the three joint groups in a patient with RA. We found that the sixth subgroup of patients, characterized by moderate activity with dominant involvement of large and wrist joints, demonstrated dominant destruction of wrist joints. This suggests that classifying patients with RA into appropriate subgroups would lead to prediction of patterns of joint destruction.