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TABLE 1. BASELINE CHARACTERISTICS OF THE PARTICIPANTS

Characteristic	Childhood asthma	Control 1	Adult asthma	Control 2
Total no.	639	838	641	376
Male - %	59.4	73.4	42.8	46.8
Age - year				
Mean	9.3	49.8	51.8	50.7
Range	4-15	20-75	20-75	29-72
Atopy - no./no. tested (%)	639/639		531/596	
Serum IgE - IU	1101.5±94.9		621.9±62.6	93.9±6.9
Eosinophil count - no./μl	518.1±15.0		392.4±16.0	
FVC- % of predicted value			84.0±0.84	
FEV1.0 - % of predicted value			71.1±0.88	
FEV1.0/FVC - %			66.8±0.78	

Plus-minus values are means ± standard error.

TABLE 2. GENOTYPE COUNTS, FREQUENCIES AND CASE-CONTROL ASSOCIATION TEST RESULTS

db SNP ID	Case				Control				MAF		*P* values, ORs (95% c.i.)		
Allele1/2	1/1	1/2	2/2	Sum	1/1	1/2	2/2	Sum	Case	Control	Allelic	Dominant	Recessive
Childhood atopic asthma					Control 1								
rs3806933	294	269	68	631	446	311	72	829	0.32	0.27	0.0063	0.0064	0.18
-847C/T	0.47	0.43	0.11		0.54	0.38	0.09				1.25 (1.07-1.47)	1.34 (1.09-1.64)	1.27 (0.90-1.80)
rs2289276	322	256	60	638	493	288	56	837	0.29	0.24	0.00066	0.0013	0.055
-82C/T	0.5	0.4	0.09		0.59	0.34	0.07				1.33(1.13-1.57)	1.41(1.14-1.73)	1.45(0.99-2.12)
rs2289278	418	195	25	638	537	261	38	836	0.19	0.20	0.52	0.61	0.56
1560C/G	0.66	0.31	0.04		0.64	0.31	0.05				0.94 (0.78-1.13)	0.95 (0.76-1.17)	0.86 (0.51-1.43)
Adult asthma					Control 2								
rs3806933	289	274	71	634	204	143	27	374	0.33	0.26	0.0023	0.0060	0.039
-847C/T	0.46	0.43	0.11		0.55	0.38	0.07				1.37 (1.12-1.67)	1.43 (1.11-1.85)	1.62 (1.02-2.58)
rs2289276	322	264	53	639	213	138	23	374	0.29	0.25	0.034	0.044	0.21
-82C/T	0.5	0.41	0.08		0.57	0.37	0.06				1.25 (1.02-1.53)	1.30 (1.01-1.68)	1.38 (0.83-2.29)
rs2289278	415	187	29	631	232	127	17	376	0.19	0.21	0.28	0.19	0.96
1560C/G	0.66	0.30	0.05		0.62	0.34	0.05				0.88 (0.71-1.11)	0.84(0.64-1.09)	1.02(0.55-1.88)
Combined (Mantel-Haenszel)													
rs3806933	583	543	139	1265	650	454	99	1203	0.32	0.27	0.000056	0.00013	0.022
-847C/T	0.46	0.43	0.11		0.54	0.38	0.08				1.29 (1.14-1.47)	1.37 (1.16-1.62)	1.39 (1.05-1.85)
rs2289276	644	520	113	1277	706	426	79	1211	0.29	0.24	0.000076	0.00019	0.026
-82C/T	0.50	0.41	0.09		0.58	0.35	0.07				1.3 (1.14-1.48)	1.36 (1.16-1.61)	1.42 (1.04-1.96)
rs2289278	833	382	54	1269	769	388	55	1212	0.19	0.21	0.25	0.23	0.69
1560C/G	0.66	0.30	0.04		0.63	0.32	0.05				0.92 (0.79-1.06)	0.90 (0.76-1.07)	0.92 (0.61-1.39)

P values of the two populations represent the chi square test for case-control comparisons under each

model.

Figure Legends

Figure 1. SNPs and pairwise LD map of the *TSLP* gene. (*A*) A graphical overview of polymorphisms identified in relation to the exon/intron structure of the human *TSLP* gene. Three polymorphisms were genotyped in this study. The ATG, UTR, and the ORF are shown by closed triangles, white boxes, and black boxes, respectively. (*B*) Pairwise D'/LOD (left) and r^2 (right) for all combinations of SNP pairs are shown.

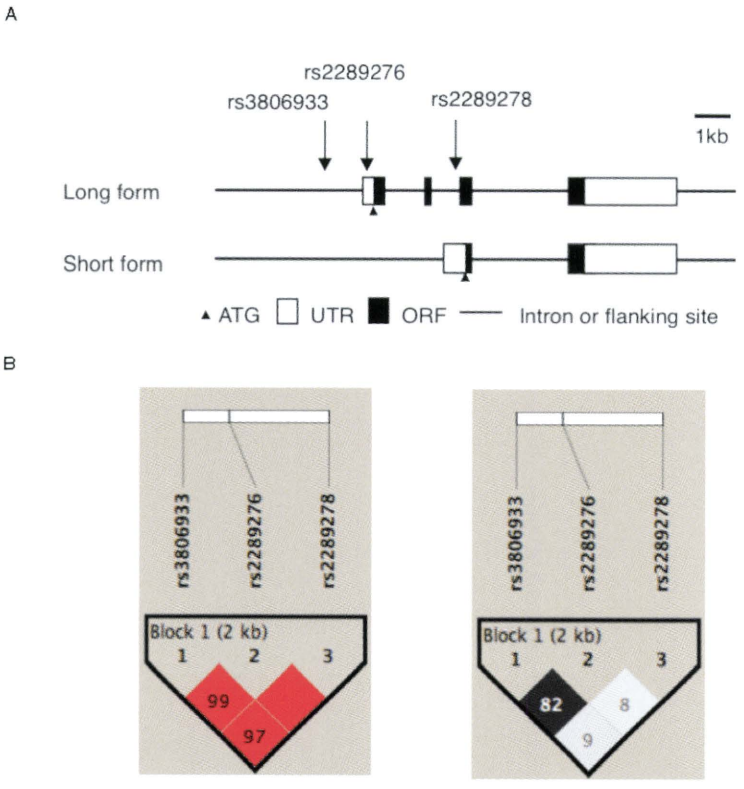
Figure 2. Relationship of *TSLP* rs3806933, rs2289276 and rs2289278 genotype with lung function in adult asthma patients. The percentage of forced expiratory volume in 1 second (FEV_1) to forced vital capacity (FVC) is plotted, and horizontal bars represent the mean for each genotype group.

Figure 3. (*A*) The DNA sequences of transcription factor-binding motifs around rs2289276 SNP. The positions of potential AP-2α binding sites are shown in the open box and the asterisks (*) represent SNPs. (*B*) Binding affinity of transcription factors to oligonucleotides in vitro. NHBE were stimulated with or without 10μg/ml poly(I:C) for 1h. Proteins interacting with the double-stranded oligonucleotides were precipitated and analyzed by immunoblotting with the indicated antibodies. Three independent experiments were performed with similar results.

Figure 4. Suppression of TSLP production by SAL and DEX in NHBE. (*A*) Quantitative RT-PCR assay of the *TSLP*. $P < .001$, by Student's t-test. (*B*) Effects of DEX and SAL on TSLP protein production in NHBE stimulated by poly(I:C). The concentrations of TSLP in supernatants were measured by ELISA. *, not detectable.

Figure 5. (*A*) Luciferase constructs. The gray box indicates the NF-κB regulatory region. (*B*) Effects of dexamethasone and salmeterol on luciferase transcriptional activities of haplotypes of the long form of *TSLP* in NHBE. Data represent mean ± SD and are from three experiments in triplicate. $*P < 0.0001$ and $\dagger P = 0.0021$ by the Bonferroni-Dunn test with two-factor factorial ANOVA.

Figure 1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rs3806933
rs2289278
1kb
Long form
Short form
ATG
UTR
ORF
Intron or flanking site
B
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rs2289278
Block 1 (2 kb)
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2
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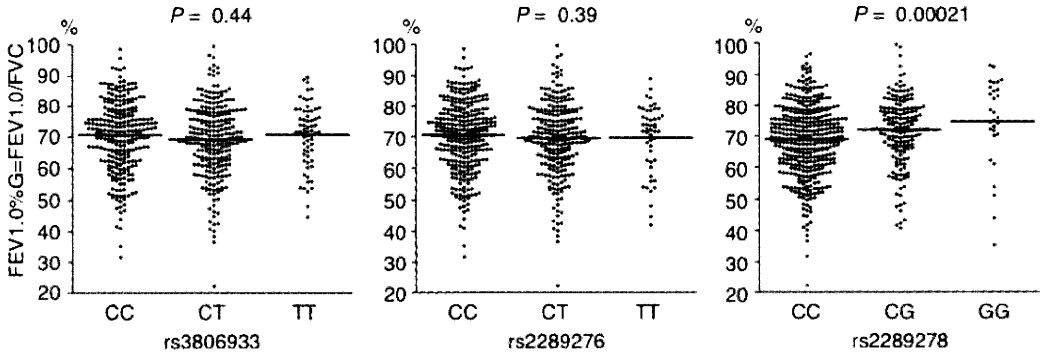
Block 1 (2 kb)
1
2
3
82
8
9

Figure 2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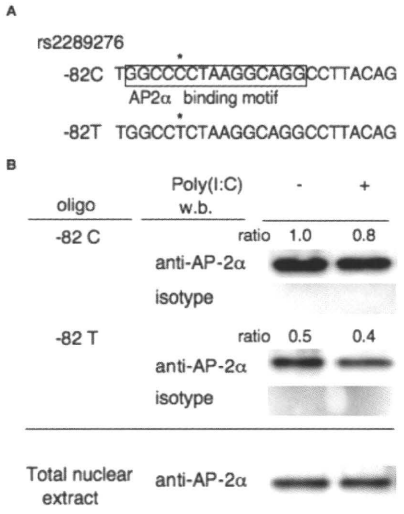
rs3806933
rs2289276
rs2289278

Figure 3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AP2α binding motif
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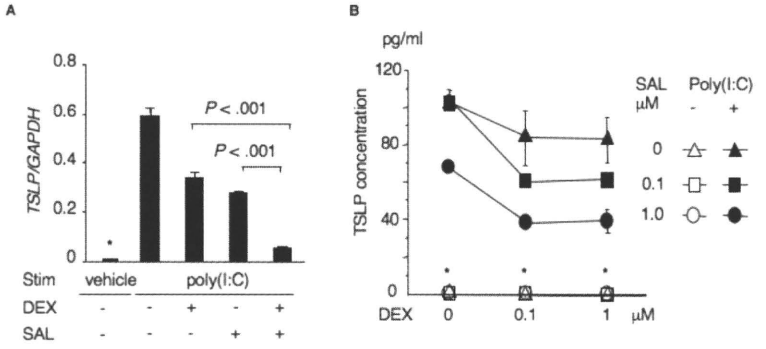
oligo
Poly(I:C)
w.b.
-
+
-82 C
ratio 1.0 0.8
anti-AP-2α
isotype
-82 T
ratio 0.5 0.4
anti-AP-2α
isotype
Total nuclear extract
anti-AP-2α

Figure 4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TSLP/GAPDH
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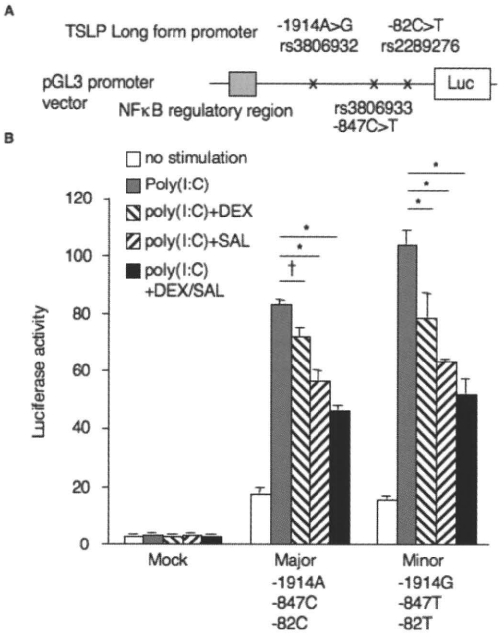
P < .001
Stim
vehicle
poly(I:C)
DEX
SAL
B
pg/ml
TSLP concentration
120
80
40
0
SAL
μM
Poly(I:C)
0
0.1
1.0
DEX
0
0.1
1
μM

Figure 5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rs3806932
-82C>T
rs2289276
pGL3 promoter
vector
NFκB regulatory region
rs3806933
-847C>T
Luc
B
no stimulation
Poly(I:C)
poly(I:C)+DEX
poly(I:C)+SAL
poly(I:C)
+DEX/SAL
Luciferase activity
120
100
80
60
40
20
0
Mock
Major
-1914A
-847C
-82C
Minor
-1914G
-847T
-82T

Online Data Supplement

TSLP Promoter Polymorphisms are Associated with Susceptibility to Bronchial Asthma

Michishige Harada, Tomomitsu Hirota, Aya I. Jodo, Yuki Hitomi, Masafumi Sakashita, Tatsuhiko Tsunoda, Takehiko Miyagawa, Satoru Doi, Makoto Kameda, Kimie Fujita, Akihiko Miyatake, Tadao Enomoto, Emiko Noguchi, Hironori Masuko, Tohru Sakamoto, Nobuyuki Hizawa, Yoichi Suzuki, Shigemi Yoshihara, Mitsuru Adachi, Motohiro Ebisawa, Hirohisa Saito, Kenji Matsumoto, Toshiharu Nakajima, Rasika A. Mathias, Nicholas Rafaels, Kathleen C. Barnes, Blanca E. Himes, Qing Ling Duan, Kelan G. Tantisira, Scott T. Weiss, Yusuke Nakamura, Steven F. Ziegler, and Mayumi Tamari

Study Subjects and Genotyping

All subjects with bronchial asthma were diagnosed according to the criteria of the National Institutes of Health (National Heart, Lung, and Blood Institute, National Institutes of Health, 1991) by doctors who were specialists for asthma (20-22). We recruited 639 subjects with childhood atopic asthma from the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Dokkyo University School of Medicine, National Research Institute for Child Health & Development, and National Sagamihara Hospital. A total of 641 subjects with adult asthma were recruited from the Miyatake Asthma Clinic and Showa University School of Medicine. After exclusion of individuals who had been diagnosed with asthma, atopic dermatitis or nasal allergies by physicians' interviews, a total of 838 healthy individuals were recruited as described (20-22). There was an age difference between the childhood asthma and control groups. We performed linear regression analysis between age and the genotypes of the three SNPs (Table E4), and found no evidence of association between age and genotype. A total of 376 healthy individuals who had never been diagnosed with asthma, atopic dermatitis or nasal allergies were recruited during their annual health checkup as a second control group in the University of Tsukuba. The serum IgE level was $\log_{10}$-transformed before analysis. A recent study of Japanese population structure has shown that most Japanese individuals fall into two main clusters, Hondo and Ryukyu; the Hondo cluster includes most of the individuals from the main islands in Japan (37). All subjects in this study were Japanese and were recruited from Hondo area. We confirmed no obvious population stratification in subjects with adult asthma and healthy individuals (22). Genotyping completion rates were ≥99% for all three SNPs. All SNPs were in Hardy Weinberg equilibrium in the two control groups.

Statistical Analysis

We resequenced the *TSLP* gene using 24 subjects with asthma and 12 control volunteers (10). We selected three Tag SNPs for association study by using the LD map shown in a recent study (10). The three Tag SNPs captured all seven of the SNPs with a mean r^2 of 1.00 among the 36 Japanese subjects (10). Rs3806933 was in complete LD with rs3806932, rs2289277, and rs10073816 (D' = 1.00 and r^2 = 1.00). Rs2289276 was in complete LD with rs 11466741 (D' = 1.00 and r^2 = 1.00) (10) (Figure

1B). Although we searched the HapMap release 27 database to find whether any additional information was available, the database did not show any novel SNP in the *TSLP* gene.

To test the association between *TSLP* variants and bronchial asthma, we compared differences in the allele frequency and genotype distribution of each polymorphism between case and control subjects by using a contingency chi-square test. We further investigated associations between asthma-related phenotypes (eosinophil count, serum total IgE, lung functions and disease severity) and variants within patients with asthma (22). Serum total IgE, eosinophil counts and lung functions (% of predicted FEV_1, % of predicted FVC and FEV_1:FVC) were analyzed as quantitative levels by the Kruskal-Wallis test. The correlations between the lung functions and the alleles of SNPs were evaluated with the Jonckheere-Terpstra test. We surveyed associations between the SNPs and disease severity as described (23). The clinical severity of adult asthma was classified according to the criteria of the National Institutes of Health/Global Initiative for Asthma 2002. The distribution of subjects was as follows: step 1, mild intermittent 2.2% (14 individuals); step 2, mild persistent 51.4% (327 individuals); step 3, moderate persistent 28.8% (183 individuals); and step 4, severe persistent 17.6% (112 individuals). We divided the subjects with asthma into two groups, steps 1 and 2 versus steps 3 and 4 by sample size (53.6 vs. 46.4%).

Biotinylated Oligonucleotide Precipitation Assay

NHBE cells were lysed on ice for 15 min and insoluble material was removed by centrifugation as described (10). The supernatant was diluted 1:3 with buffer, and the lysate was preabsorbed using ImmunoPure streptavidin-agarose beads (Pierce, Rockford, IL) for 1 h. The sample was then incubated with 3 μg of biotinylated double-stranded oligonucleotides, together with 3 μg of poly(dI-dC) for 1 h. Biotinylated DNA-protein complexes were recovered using streptavidin-agarose beads for 1 h, and separated on SDS-polyacrylamide gels. AP-2α was detected by immunoblotting with an anti-AP-2α antibody. The band intensity was determined by densitometry, and the intensity ratio of each band to that of -82C without poly(I:C) were shown (Figure 3B).

Quantitative Real-Time RT-PCR

NHBE cells were purchased and maintained using medium kits (BulletKit) (Cambrex, East Rutherford, NJ). Cells were stimulated with 10 μg/ml poly(I:C) (InvivoGen, La Jolla, CA). Poly(I:C) stimulated NHBE cells were cultured with dexamethasone (ICN Biomedicals, Costa Mesa, CA) and/or salmeterol (TOCRIS Inc., Ellisville, MO). The expression of *TSLP* was determined by real-time quantitative RT-PCR, and the amounts of cDNA were standardized by quantification of the housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) in all experiments as described (10).

Luciferase Assay

The reporter plasmids for TSLP were generated using the reporter gene pGL3-promoter vector (Promega, Madison, WI) as described (10). NHBE (5×10^4/well) were transfected with these reporter constructs (500ng) and pRL-TK Renilla luciferase vector (10ng) as a normalization control using FuGENE 6 transfection reagent (Roche

Diagnostics, Indianapolis, IN) in a 12-well plate. Twenty-four hours later, transfected cells were stimulated with 10 μg/ml poly(I:C) for 4 h before luciferase assays (Promega). Dexamethasone (1 μM) and/or salmeterol (1 μM) were added to the medium 0.5 h before the stimulation with poly(I:C).

Table E1. Genotype counts and case-control association test results

db SNP ID	Case				Control				MAF		*P* values, ORs (95% c.i.)		
Allele1/2	1/1	1/2	2/2	Sum	1/1	1/2	2/2	Sum	Case	Control	Allelic	Dominant	Recessive
Childhood atopic asthma					Control 1								
rs3806933	294	269	68	631	446	311	72	829	0.32	0.27	0.0063	0.0064	0.18
-847C/T	0.47	0.43	0.11		0.54	0.38	0.09				1.25 (1.07-1.47)	1.34 (1.09-1.64)	1.27 (0.90-1.80)
rs2289276	322	256	60	638	493	288	56	837	0.29	0.24	0.00066	0.0013	0.055
-82C/T	0.5	0.4	0.09		0.59	0.34	0.07				1.33 (1.13-1.57)	1.41 (1.14-1.73)	1.45 (0.99-2.12)
rs2289278	418	195	25	638	537	261	38	836	0.19	0.20	0.52	0.61	0.56
1560C/G	0.66	0.31	0.04		0.64	0.31	0.05				0.94 (0.78-1.13)	0.95 (0.76-1.17)	0.86 (0.51-1.43)
Adult asthma diagnosed in childhood					Control 2								
rs3806933	60	66	14	140	204	143	27	374	0.34	0.26	0.022	0.018	0.30
-847C/T	0.43	0.47	0.10		0.55	0.38	0.07				1.41 (1.05-1.90)	1.60 (1.08-2.37)	1.43 (0.73-2.81)
rs2289276	67	63	9	139	213	138	23	374	0.29	0.25	0.14	0.077	0.89
-82C/T	0.48	0.45	0.06		0.57	0.37	0.06				1.26 (0.93-1.71)	1.42 (0.96-2.10)	1.06 (0.48-2.34)
rs2289278	107	24	8	139	232	127	17	376	0.14	0.21	0.012	0.0012	0.56
1560C/G	0.77	0.17	0.06		0.62	0.34	0.05				0.62 (0.42-0.90)	0.48(0.31-0.75)	1.29(0.54-3.06)
Combined (Mantel-Haenszel)													
rs3806933	583	543	139	771	650	454	99	1203	0.32	0.27	0.00057	0.00047	0.11
-847C/T	0.46	0.43	0.11		0.54	0.38	0.08				1.28 (1.11-1.48)	1.39 (1.15-1.68)	1.30 (0.94-1.80)
rs2289276	644	520	113	777	706	426	79	1211	0.29	0.24	0.00025	0.00027	0.078
-82C/T	0.50	0.41	0.09		0.58	0.35	0.07				1.31 (1.13-1.52)	1.41 (1.17-1.70)	1.36 (0.95-1.94)
rs2289278	833	382	54	777	769	388	55	1212	0.18	0.21	0.083	0.052	0.91
1560C/G	0.68	0.28	0.04		0.63	0.32	0.05				0.86 (0.73-1.02)	0.83 (0.68-1.00)	0.95 (0.59-1.51)

Table E2. Haplotype frequencies of polymorphisms of the *TSLP* gene

	-847C/T	-82C/T	1560C/G	Childhood atopic		Adult	
	rs3806933	rs2289276	rs2289278	Asthma	Control 1	Asthma	Control 2
Haplotype 1	C	C	C	622 (0.49)	878 (0.53)	611 (0.48)	392 (0.52)
Haplotype 2	T	T	C	374 (0.29)	398 (0.24)	370 (0.29)	184 (0.25)
Haplotype 3	C	C	G	243 (0.19)	336 (0.20)	250 (0.20)	161 (0.21)
Haplotype 4	T	C	C	35 (0.03)	58 (0.03)	51 (0.04)	15 (0.02)
				1374	1670	1282	752

	$\chi 2$	*P* value	OR (95% c.i.)
Childhood atopic asthma			
Haplotype 1 versus others	4.067	0.044	0.86 (0.74-1.00)
Haplotype 2 versus others	11.399	0.00070	1.33 (1.13-1.57)
Adult Asthma			
Haplotype 2 versus others	4.616	0.032	1.25 (1.02-1.54)
Haplotype 4 versus others	5.901	0.015	2.04 (1.14-3.65)

Table E3. Genotype counts and case-control association test results in genome-wide association studies (GWAS).

db SNP ID	Case				Control				MAF		*P* values, ORs (95% c.i.)		
Allele1/2	1/1	1/2	2/2	Sum	1/1	1/2	2/2	Sum	Case	Control	Allelic	Dominant	Recessive
GWAS of asthma with the cases from the Childhood Asthma Management Program (CAMP) (ref. 24)													
	Case				Control								
rs3806932	128	165	65	358	272	412	162	846	0.41	0.43	0.30	0.23	0.69
-1914A/G	0.36	0.46	0.18		0.32	0.49	0.19						
rs2289276	192	145	22	359	421	354	71	846	0.26	0.29	0.14	0.24	0.18
-82C/T	0.53	0.40	0.06		0.50	0.42	0.08						
rs11466741	191	145	23	359	419	356	71	846	0.27	0.29	0.16	0.24	0.24
1117C/T	0.53	0.40	0.06		0.50	0.42	0.08						
GWAS on African-ancestry populations for asthma (ref. 25)													
	Case (African American)				Control								
rs3806932	62	204	196	462	63	221	184	468	0.65	0.63	0.48	0.99	0.34
-1914A/G	0.13	0.44	0.42		0.13	0.47	0.39						
rs2289276	331	119	13	463	309	140	16	465	0.16	0.18	0.10	0.10	0.58
-82C/T	0.71	0.26	0.03		0.66	0.30	0.03						
rs11466741	229	192	41	462	218	209	42	469	0.30	0.31	0.46	0.35	0.97
1117C/T	0.50	0.42	0.09		0.46	0.45	0.09						
	Case (African Caribbean)				Control								
rs3806932	38	167	173	378	54	199	201	454	0.68	0.66	0.47	0.40	0.67
-1914A/G	0.10	0.44	0.46		0.12	0.44	0.44						
rs2289276	278	92	11	381	315	130	14	459	0.15	0.17	0.21	0.17	0.89
-82C/T	0.73	0.24	0.03		0.69	0.28	0.03						
rs11466741	198	150	31	379	239	183	37	459	0.28	0.28	0.99	0.96	0.95
1117C/T	0.52	0.40	0.08		0.52	0.40	0.08						

1/1, homozygous of major allele; 1/2, heterozygous; 2/2, homozygous of minor allele in the Japanese population.

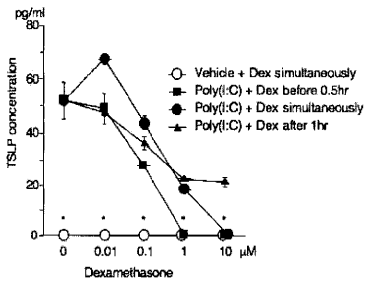
Table E4. Correlation between age and genotypes of the three SNPs

Characteristic	Childhood atopic asthma	Control 1	Adult asthma	Control 2	Total
Total no.	639	838	641	376	2494
rs3806933					
Spearman's rho value	0.068	-0.011	0.018	-0.026	0.036
P value	0.097	0.76	0.65	0.62	0.072
rs2289276					
Spearman's rho value	-0.067	0.001	0.006	0.032	-0.038
P value	0.11	0.98	0.87	0.54	0.063
rs2289278					
Spearman's rho value	-0.008	0.007	0.052	-0.015	0.011
P value	0.85	0.85	0.19	0.77	0.58

Spearman's rho (nonparametric correlation coefficient) values are shown with *P* values.

Supplemental Figure Legends

Figure E1. TSLP production in NHBE. Cells were stimulated with or without poly(I:C), and the concentrations of TSLP in supernatants 24 h after stimulation were measured by ELISA. DEX was added at the indicated time points and doses. Data are representative of three independent experiments and represent mean ± SD, and similar results were obtained using NHBE from three individuals.


pg/ml
80
60
40
20
0
TSLP concentration
Vehicle + Dex simultaneously
Poly(I:C) + Dex before 0.5hr
Poly(I:C) + Dex simultaneously
Poly(I:C) + Dex after 1hr
0
0.01
0.1
1
10 μM
Dexamethasone

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Prevalence of Allergic Rhinitis and Sensitization to Common Aeroallergens in a Japanese Population

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Key Words
Aeroallergen · Allergic rhinitis · Dust mite · Specific human IgE · Japanese cedar pollen

Abstract
Background: Allergic rhinitis (AR) is recognized as a major health problem worldwide, and its prevalence depends on the age range of the subjects. The aims of this study were to determine the current prevalence of AR, effects of age on the prevalence of IgE sensitization to inhalant allergens, and serum total IgE levels in Japanese subjects. ***Methods:*** We conducted a survey of 1,540 subjects between 20 and 49 years of age in 2006 and 2007 and examined the prevalence of AR and sensitization to 7 common aeroallergens. We measured serum total IgE and specific IgE to 7 aeroallergens. AR was determined based on symptoms, predominantly in the nose and eyes, caused by aeroallergens as mentioned in a questionnaire and sensitization to any of the 7 aeroallergens as assessed by measurement of serum specific IgE. ***Results:*** The prevalence of AR was 44.2% (681 of the 1,540 subjects) and there was no difference among age decades. Of the 1,540 subjects, 1,073 (69.7%) were sensitized to at least 1 of the 7 aeroallergens. The most common allergen in AR was Japanese cedar pollen (89.6%, 610 of the 681 with AR) in all the age decades examined. The sensitization rate to mites was significantly higher in the younger subjects. ***Conclusion:*** Our data suggest that the prevalence of AR between 20 and 49 years of age has increased by nearly 10% during the last 10 years. Cedar pollen and mites were predominant allergen sources among the 7 aeroallergens in the Japanese population.

Introduction

Allergic rhinitis (AR), the most common type of rhinitis, is a heterogeneous disorder that significantly impairs the patient's quality of life, and its prevalence has markedly increased in recent decades [1, 2]. Epidemiologic and serological studies have provided valuable information to develop effective strategies for the prevention and treatment of the disease [3–6]. Japanese cedar

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pollinosis (JCP) is a common allergic disease, and the increase in its prevalence is a major public health problem in Japan [7]. Several epidemiologic studies have been conducted on JCP [8–11]. Sakurai et al. [9] reported the prevalence and risk factors of AR and JCP among 2,307 Japanese men; the prevalence rates of AR, seasonal rhinitis and JCP were 35.5, 28.8 and 11.0%, respectively, in 1998. Kaneko et al. [10] conducted a meta-regression analysis of 38 population-based surveys in Japan. The prevalence of JCP among adolescents in the general population was estimated at 28.7% in metropolitan areas and 24.5% in urban areas in the year 2004. The study also reported that the prevalence of JCP increased 2.6-fold between 1980 and 2000. To monitor the prevalence of sensitization is useful for understanding AR and developing preventive measures.

In AR, an IgE-mediated response to allergens is triggered and characterized by type-2-helper-T-cell-dependent inflammation [12]. Allergen-specific IgE is a critical factor in the mechanism of AR. Serum allergen-specific IgE results closely correlate to those of skin tests and nasal challenges. Allergen-specific IgE tests are highly specific and sensitive. One of their advantages is that drugs and skin diseases do not influence the measurement [1].

Sensitization is an important risk factor for developing allergic disease [13]. Epidemiological investigation of AR is important to clarify its etiology and develop appropriate preventive and therapeutic techniques. There have been few epidemiological studies on the age effect on the prevalence of AR and IgE sensitization to inhalant allergens, and serum total IgE levels in Japanese subjects. Therefore, we conducted an epidemiological study on a total of 1,540 subjects aged 20–49 years. The protocol comprised a questionnaire, measurement of total serum IgE antibodies and allergen-specific IgE antibodies against 7 aeroallergens in 2006 and 2007. The major findings of this study are the prevalence of allergic sensitization and AR, the age effect on them, and total serum IgE and AR, and the related age effect.

Material and Methods

Study Subjects

A total of 1,540 subjects were recruited from residents of Eiheiji-cho and the cities of Fukui, and Echizen in Fukui prefecture, in the central Hokuriku area of Japan in May and June of both 2006 and 2007. In that area, Japanese cedar pollen counts are at the average level of the islands of Honshu, Shikoku and Kyushu [7]. The 1,540 subjects were workers of 4 hospitals and students of nursing and medical colleges in the University of Fukui. The number of females was higher than that of males (mean age, 32.1 years; range, 20–49 years; male:female ratio, 1.0:2.40; mean serum IgE level, 233.8 IU/ml; median serum IgE level, 73.5 IU/ml). The participants were recruited during their annual health check-up in 2006 or 2007; 13 subjects did not agree to participate in this survey. Reasons for nonparticipation were lack of interest or time. All of the 1,540 participants agreed to measurement of serum total IgE and specific IgE to 7 aeroallergens and to answer a questionnaire. Blood collection and the questionnaire survey were performed at the same time after informed consent was received. We did not conduct a follow-up survey in this study. The diagnosis of AR was confirmed by seasonal or perennial symptoms of rhinitis consisting of any combination of the following: nasal itching, sneezing, discharge and stuffiness caused by inhalation of aeroallergens, reported on a questionnaire. All of the subjects with AR were also positive for serum-specific IgE to 1 or more of the 7 aeroallergens. All individuals were unrelated Japanese individuals and gave written informed consent to participate in the study according to the rules of the ethics committees of the Faculty of Medical Science, University of Fukui and the Institute of Physical and Chemical Research (RIKEN).

Measurement of Serum Levels of Specific IgE Antibodies

Specific IgEs to 7 aeroallergens, *Cryptomeria japonica*, *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farinae* (Der f), *Dactylis glomerata*, *Ambrosia artemisiifolia*, *Candida albicans* and *Aspergillus fumigatus* were measured with a Pharmacia CAP System (Pharmacia CAP, Upsala, Sweden) (table 1). Allergen sensitization was classified as positive if the allergen-specific serum IgE level was above 0.7 (CAP RAST score of 2).

Statistical Analysis

To clarify the age-specific prevalence of AR and sensitization to the 7 aeroallergens examined, patients were divided into 3 age groups, the 20s (20 to <30 years), 30s (30 to <40 years) and 40s (40 to <50 years). We then compared differences in frequencies of sensitization to each of the 7 aeroallergens among these age groups by using the Kruskal-Wallis test and then by individual testing using the Mann-Whitney U test if significant. Serum total IgE was analyzed at a quantitative level, and log-transformed individual serum IgE levels were used in the figures. Correlations of total IgE levels and age were analyzed by Spearman's test. $p < 0.05$ was considered statistically significant. Logistic regression analysis was implemented for the AR and sensitization to assess the effects of gender, age and total serum IgE (SPSS 14.0J, SPSS, Inc., Chicago, Ill., USA).

Results

Prevalence of Allergic Sensitization and AR

Positive sensitization refers to an allergen-specific serum IgE level >0.7 (CAP RAST score of 2). The prevalence of allergic sensitization to each allergen tested is presented in table 1. Of the 1,540 subjects, 1,073 (69.7%) exhibited positive sensitization to at least 1 aeroallergen (fig. 1). A total of 467 of the 1,540 subjects (30.3%) showed

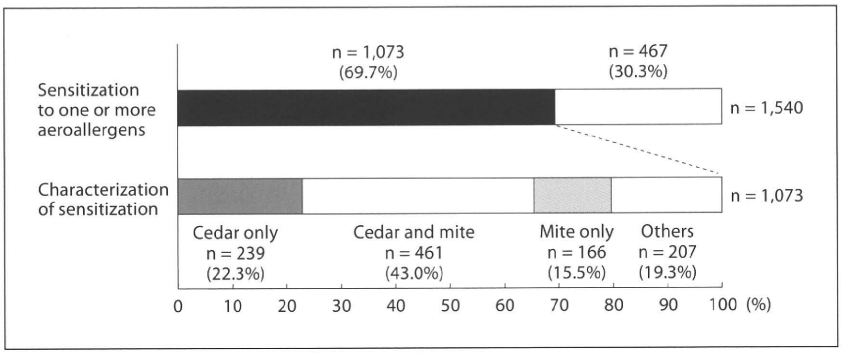

Fig. 1. The prevalence of sensitization to 7 test aeroallergens and characterization of sensitization.

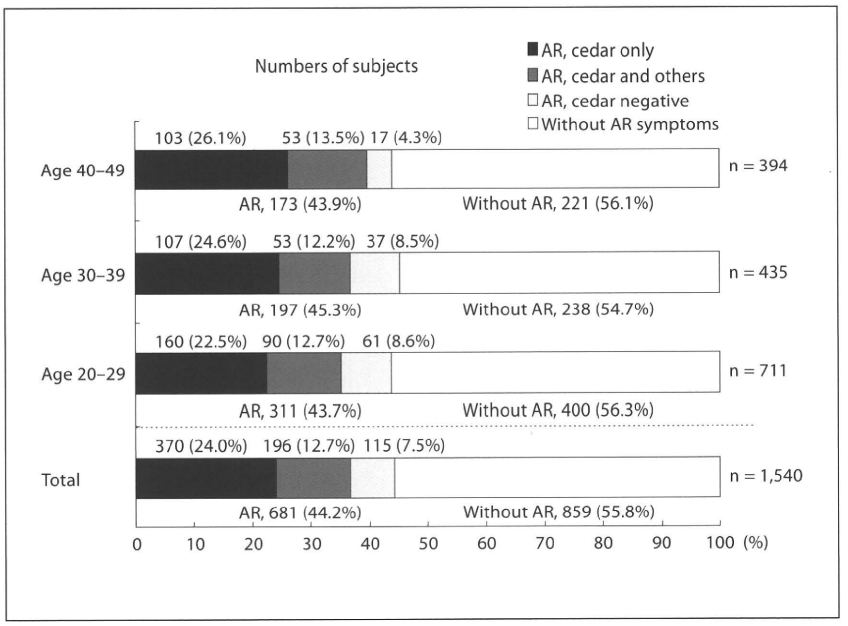

Fig. 2. Age effects on the prevalence of AR and sensitization to Japanese cedar pollen.

no sensitization to any of the 7 aeroallergens examined (fig. 1). Seven hundred subjects (45.3%) were sensitized to *C. japonica*, (Japanese cedar, JC) pollen, thus accounting for 65.3% of the 1,073 subjects with positive sensitization to aeroallergens. Of the 1,073 subjects, 627 (58.5%) were sensitized to mites. Thus, JC pollen and mites were the two predominant aeroallergens among the 7 tested aeroallergens (fig. 1).

Of the 1,540 participating subjects, 681 (44.2%) had symptoms of AR at the time of the survey (fig. 2). The prevalence of JCP was 36.7% (566 of the 1,540 subjects) in this study (fig. 2). The positive rates for specific IgE antibodies to Japanese cedar pollen were 89.6% (610 of 681) in the AR group and 28.5% (245 of 859) in the no-symptom group (fig. 3). Of the 681 AR subjects, 167 (24.5%) were sensitized to only Japanese cedar pollen (fig. 3).

Age Effect on the Prevalence of Allergic Sensitization and AR

We found significant associations between the allergic sensitization to the 7 aeroallergens and the age groups (table 1) ($p = 0.0019$ by the Kruskal-Wallis test). More subjects were sensitized to Japanese cedar pollen than to any other of the 7 tested allergens in each age group (table 1). The sensitization rates to Japanese cedar pollen were 59% (421 of 711 subjects), 52% (226 of 435) and 53% (208 of 394) for subjects in their 20s, 30s and 40s, respectively. We found a significant association between sensitization to Japanese cedar pollen and the age range of the subjects ($p = 0.015$ by the Mann-Whitney U test) (table 2). The sensitization rate against mites, Der p and/or Der f, was higher for those in their 20s (50%, 355 of 711 subjects), than for those in their 30s (41%, 179 of 435) and 40s

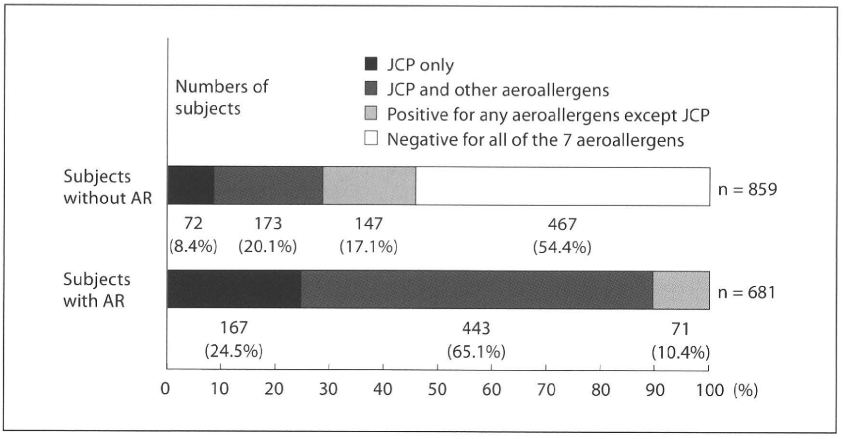

Fig. 3. Prevalence of AR and sensitization to Japanese cedar pollen.

Table 1. Prevalence of sensitization to 7 aeroallergens according to age group

	Total (n = 1,540)	20s (n = 711)	30s (n = 435)	40s (n = 394)
Cryptomeria japonica	855 (56)	421 (59)	226 (52)	208 (53)
Dermatophagoides pteronyssinus	625 (41)	345 (49)	174 (40)	106 (27)
Dermatophagoides farinae	622 (40)	342 (48)	168 (39)	112 (28)
Dactylis glomerata	352 (23)	198 (28)	90 (21)	64 (16)
Ambrosia artemisiifolia	137 (9)	67 (9)	45 (10)	25 (6)
Candida albicans	82 (5)	43 (6)	24 (6)	15 (4)
Aspergillus fumigatus	34 (2)	25 (4)	8 (2)	1 (0.3)

Figures in parentheses are percentages.

Table 2. Age effects on sensitization to JCP, dust mites and *Dactylis glomerata*

Aeroallergen	Sensitization	20s (n = 711)	30s (n = 435)	40s (n = 394)	p value
Cryptomeria japonica	positive	421 (59)	226 (52)	208 (53)	0.015
	negative	290 (41)	209 (48)	186 (47)	
Dust mites	positive	355 (50)	179 (41)	115 (29)	3.9×10^{-11}
	negative	356 (50)	256 (59)	279 (71)	
Dactylis glomerata	positive	198 (28)	90 (21)	64 (16)	4.8×10^{-6}
	negative	513 (72)	345 (79)	330 (84)	

Figures in parentheses are percentages. p value as obtained by the Mann-Whitney U test.

(29% 115 of 394), ($p < 0.001$ by the Mann-Whitney U test) (table 2). The prevalence of sensitization to *D. glomerata* was also higher in those in their 20s (28%, 198 of 711 subjects) than in those in their 30s (21%, 90 of 435) and 40s (16%, 64 of 394) ($p < 0.001$ by the Mann-Whitney U test) (table 2). AR was confirmed in 311 of the 711 subjects (43.7%) in their 20s, 197 of the 435 (45.3%) in their 30s and 173 of the 394 (43.9%) in their 40s (fig. 2). There was no significant difference in the prevalence of AR among the age groups.

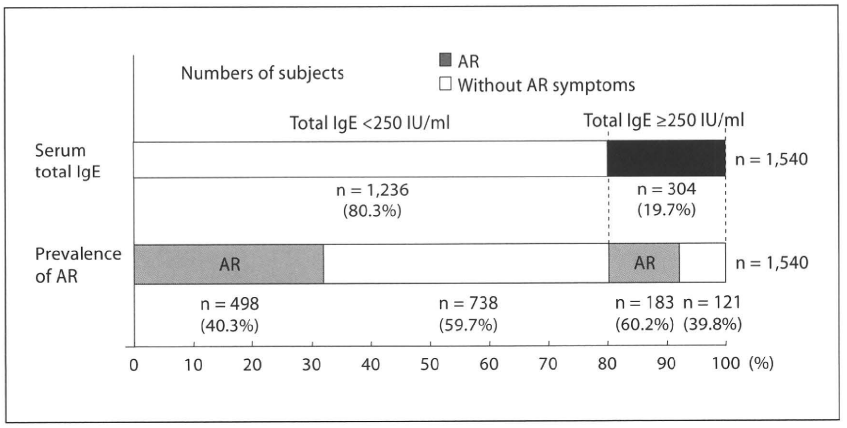

Fig. 4. Serum total IgE levels and prevalence of AR.

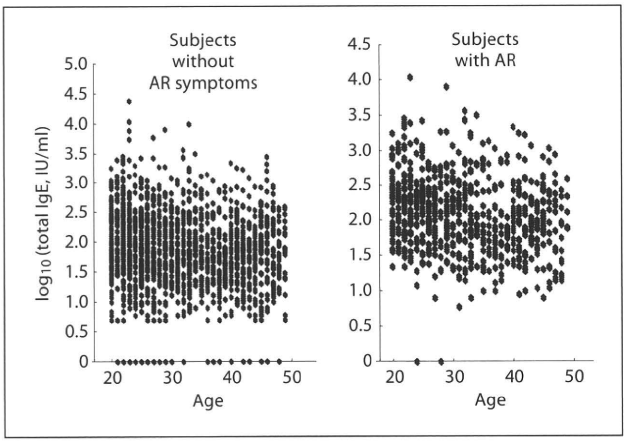

Fig. 5. Age effects on serum total IgE levels in subjects with AR and non-AR.

Total Serum IgE and AR, Sensitization, and Age Effect

There were 304 subjects (19.7%) who had high total IgE levels (≥250 IU/ml), and the prevalence of AR in this group was 60.2% (183 of the 304). However, the prevalence of AR of subjects with normal total IgE (<250 IU/ml) was 40.3% (498 of 1,236) (fig. 4).

The serum total IgE level was analyzed at a quantitative level (fig. 5). The means of $\log_{10}$ [total IgE (IU/ml)] and standard deviations of all 1,540 subjects, subjects without AR and subjects with AR were 1.87 [= $\log_{10}$ (74.1 IU/ml)] ± 0.65, 1.69 [= $\log_{10}$ (49.0 IU/ml)] ± 0.67 and 2.09 [= $\log_{10}$ (123.0 IU/ml)] ± 0.53, respectively.

We investigated the correlation between this level and age using Spearman's rank correlation coefficient (fig. 5). Although we could not find any significant correlation between the serum total IgE level and the age range of the 1,540 subjects, an inverse correlation was found between the total IgE level and age in the AR group ($r_s = -0.21$, $p < 0.01$) (fig. 5). Total IgE levels were higher in younger subjects than in older subjects in the AR group. The results of the stepwise logistic regression analysis for positive sensitization to 1 or more of the 7 aeroallergens showed significant effects of total IgE (Wald statistic = 153.5, d.f. = 1, $p < 0.001$) and age (Wald statistic = 9.5, d.f. = 1, $p = 0.002$), but no effect of gender. There was no significant effect of age, gender, or total IgE on AR by logistic regression analysis.

Discussion

Estimates of the latest prevalence provide valuable information to develop effective strategies for the prevention and treatment of disease. We conducted an epidemiologic survey of AR and examined the sensitization rates against 7 aeroallergens by measuring the serum-specific IgE of 1,540 subjects aged between 20 and 49 years in a Japanese population in 2006 and 2007. The population aged between 20 and 49 years represented 38.8% of the population of Japan in 2008 according to current population estimates by the Ministry of Internal Affairs and Communications (http://www.stat.go.jp/english/data). We also examined the role of age effects on the prevalence. In this study, 681 of the 1,540 subjects (44.2%) were diagnosed as having AR. Increases in prevalence of AR and asthma have been reported by studies of relatively large populations in the United States, Great Britain, Australia and New Zealand, with cross-referenc-