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ORIGINAL ARTICLE

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An association study of asthma and related phenotypes with polymorphisms in negative regulator molecules of the TLR signaling pathway

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Abstract Although associations between endotoxin exposure or respiratory infection and asthma have been recognized, the genetic effects in these conditions are unclear. Toll-like receptors (TLRs) play an essential role in innate host defense and in the control of adaptive immune responses. IL-1R-associated kinase-M (IRAK-M) and single immunoglobulin IL-1R-related molecule (SIGIRR) negatively regulate TLR-signaling pathways. To investigate whether polymorphisms in these genes were associated with asthma or asthma-related phenotypes, we screened these genes for polymorphisms by direct sequencing of 24 asthmatics and identified 19 variants in *IRAK-M* and 12 variants in *SIGIRR*. We next conducted linkage disequilibrium mapping of the genes, and examined the association of polymorphisms and haplotypes using 391 child patients with asthma, 462 adult patients with asthma, and 639 controls. None of the alleles or haplotypes of *IRAK-M* and *SIGIRR* were associated with asthma susceptibility or asthma-related phenotype. Our results indicate that polymorphisms in *IRAK-M* and *SIGIRR* are not likely to be associated with the development of asthma in the Japanese population.

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Introduction

Toll-like receptors (TLRs) are pattern-recognition receptors (PRRs) that play an essential role in activation of the innate immune system, which in turn activates adaptive immunity (Medzhitov 2001; Akira and Takeda 2004). The role of TLR proteins in asthma has been intensively studied (Basu and Fenton 2004).

TLR2 and TLR4 ligands activate IL-1R-associated kinase (IRAKs) and TNF receptor-associated factor (TRAF) 6 and induce expression of inflammatory cytokines, and IRAK-M prevents the dissociation of the IRAK1–IRAK4 complex from myeloid differentiation primary response gene (MyD) 88, thereby inhibiting the TLR signaling pathway (Kobayashi et al. 2002; Janssens and Beyaert 2003). IRAK-M regulates TLR signaling and innate immune homeostasis, because IRAK-M-deficient macrophages produce enhanced amounts of inflammatory cytokines on TLR stimulation and bacterial challenge, and increased inflammatory responses to bacterial infection are observed in IRAK-M-deficient mice (Kobayashi et al. 2002). Reduced endotoxin tolerance in IRAK-M-deficient cells has also been reported (Kobayashi et al. 2002). Furthermore, *IRAK-M* is located in 12q14.2, one of the most consistently replicated regions linked to asthma in diverse populations, wherein Yokouchi et al. (2000) mapped a locus linked to mite-sensitive atopic asthma susceptibility in the Japanese population by sib-pair analysis.

Single immunoglobulin IL-1R-related (SIGIRR) molecules, membrane-bound molecules that contain a TIR domain, have recently been shown to be involved in the negative regulation of TLR signaling (Wald et al. 2003). After TLR stimulation, SIGIRR interact transiently with TLR4, TRAK1, and TRAF6. SIGIRR are highly expressed in many epithelial cell lines, but not expressed in primary macrophages, fibroblasts, and endothelial cells (Wald et al. 2003). The high expression of SIGIRR in epithelial cells indicates they may regulate the immune response in cells that are continually exposed to microorganisms, for example lung epithelial cells. Furthermore, SIGIRR-deficient mice were found to be highly sensitive to LPS-induced endotoxic shock (Wald et al. 2003). SIGIRR therefore act as an inhibitory factor in TLR signaling, which may be essential for regulating the detrimental effects of innate immunity, as occurs in chronic inflammation.

To investigate the relationship between the genetic variants of these three genes and asthma or asthma-related phenotypes, we searched the genes for isolated polymorphisms, performed linkage disequilibrium (LD) mapping, and conducted a genetic association study with regard to the LD pattern.

Materials and methods

Subjects

All patients with asthma were diagnosed according to the criteria of the National Institutes of Health (National Heart, Lung, and Blood Institute, National Institutes of Health 1991). Improvement in their FEV_1 measurement was at least 12% in childhood asthma and 20% in adult asthma after β_2-agonist inhalation (National Heart, Lung, and Blood Institute 1991; Hasegawa et al. 2004; Kamada et al. 2004). Diagnosis of atopic asthma was based on one or more positive skin-scratch-test responses to a range of seven common aeroallergens in the presence of a positive histamine control and a negative vehicle control. The seven aeroallergens were house dust, *Felis domesticus* dander (Feld), *Canis familiaris* dander, *Dactylis glomerata*, Ambrosia, *Cryptomeria japonica*, and *Alternaria alternata*. We recruited 391 children with asthma (mean age 9.3, 4–15 years; male:female ratio = 1.43:1.0; mite RAST positive 81.6%; atopic asthma 92%) and 462 adults with asthma (mean age 50.1, 20–75 years; male:female ratio = 1.0:1.35; atopic asthma 91.2%). For children with asthma, we recorded their age, sex, mite-specific IgE positive status, serum total IgE level, eosinophil count, clinical severity, and incidence of atopic dermatitis. Specific IgE was considered positive when values exceeded 0.35 U mL^{-1} (RAST score ≥1). The severity of childhood asthma was defined according to the amount of therapy required to control symptoms at the time of entry into the study. The grades were: grade 1, β stimulants only; grade 2, sodium cromoglycate and/or theophylline; grade 3, inhaled beclomethasone, 400 μg day^{-1} or less; grade 4, inhaled beclomethasone of more than 400 μg day^{-1}. All subjects with atopic dermatitis were diagnosed by dermatology specialists. For adults with asthma, we recorded their age, sex, serum total IgE level, eosinophil count, and clinical severity. The severity of adult asthma was classified according to the system of the National Heart, Lung, and Blood Institute (1997). The serum IgE levels was $\log_{10}$-transformed before analyses. The means of $\log_{10}$[total IgE (tIgE) (IU mL^{-1})] of patients were 2.63 [$=\log_{10}$(426.6 IU mL^{-1})] in childhood asthma and 2.34 [$=\log_{10}$(218.8 IU mL^{-1})] in adult asthma. In this study, "high IgE" and "high eosinophil count" levels were defined as those values in the 75th percentile or higher for total IgE and eosinophil count (%). The 75th percentile values of $\log_{10}$(tIgE) in patients were 3.06 [$=\log_{10}$(1,148 IU mL^{-1})] in childhood asthma and 2.71 [$=\log_{10}$(512.9 IU mL^{-1})] in adult asthma. The 75th percentile values of eosinophils in patients were 9.9 (%) in childhood asthma and 8.0 (%) in adult asthma. A total of 639 healthy

Table 1 Primers for the SNPs survey in this study

IRAK-M	F1	GCA GGC CTT TCT GAT TGC TT	R1	CAG AAA AGA CAC CAA ATC AGC
	F2	AGA AGT AAT GAC ACC GCT AG	R2	TAC ATT GCG AAC CCA GTG AG
	F3	CAG AAG GCA GGT GAA TAT ATT C	R3	AGA CAA AGG GAA GAA TTA GGC
	F4	CAA CTA CTT ATG TTT TAA GTG AAC	R4	CAG TGC AAC AGA GTG CAA CC
	F5	ACT TTG ACT GAC TAT GAC ATT G	R5	TCA GAT CTA GTG GCA AAG ACT
	F6	GTA GAG CAA TGC TGA AGG TC	R6	GCT AAG AAG GAA CAT CAC CAT
	F7	CTC TGT GGA ATG GTG GGA AC	R7	TGA CCC TCT TTA ACA AAG TCC
	F8	CTA GCT GTC ATG GGA TTG TC	R8	GAC TCT CAG ACT CAG GAG TG
	F9	TGG AAA GCA AAT CTG TGT CTG	R9	CTG TGT CAC GCT ATG GTG A
	F10	AGC AGA AGG AAA CCC ATC TG	R10	CAC CTA ACC TAC CGA ACA TC
	F11	AGA ATG TTC TCC AGT TCA TGG	R11	AGA GAT AGG TGC CAG GA TGA
	F12	GAC TCA TTG ATT TCC TGT TAG C	R12	AAT ATT CCC TGC AAA CTG CTC
	F13	AAA TAA AGG GCG TTA GCT AAT C	R13	CTC TTG GCA TTG CTT ATG GAG
	F14	TAT ATA GTT CCA TCC CAG GAC	R14	CTT GAC CAG CCA TTT TCT CAG
SIGIRR	F1	TAA TCT CTC GGA TCT CAG GC	R1	TGA GGC CTT ACT CGA CAG TA
	F2	TCA TTG CCA ATG GGA TGG TC	R2	TCA GGA GTT CAG AGG GCA TT
	F3	TCT TCC ACA CCA AGG ACT TC	R3	TCA CCC AGA GTT CAA GTC AG
	F4	CAG GAA TCC CCT GTA TGT TC	R4	ATC TCT TCC CTT TCC TCC AG
	F5	TCC AGT TTT CCA TGG GCT TC	R5	TTC GCC CAC TTT CCT CCC TT
	F6	AGG TGA TCC TGG ACT TGA TG	R6	TTA CAT CAG GGT GAT GAG CC
	F7	GCT CAT GAG GGT CAG TAA AG	R7	GAA GAG AGA GGA CAC AGT GG
	F8	TGG ACA GAC ATG GTG TGA CT	R8	AAG CCA AGA GAA GTG ACC TG
	F9	TCT GAA TGA ACA CCG ACC AG	R9	TAA CCA TCT CCC ACG TGC AC
	F10	ATG GGG AGG TGG AGA TAA AC	R10	AGG TGA TGA AGA TGG GTC TG
	F11	TCA TCG TGG TGC TTT CGG AC	R11	AAG AGT CCT CAA CAC CTG GA
	F12	ACA AGG ACC CCA TGC TGA TT	R12	AAG CCG AAT CCG AAA CCT TC

individuals who had neither respiratory symptoms nor a history of asthma-related diseases (mean age 43.5, 20–75 years; male:female ratio = 2.67:1.0) were recruited by physicians' interviews about whether they had been diagnosed with asthma and/or atopy. Genomic DNAs were prepared in accordance with standard procedures. All individuals were Japanese and gave written informed consent to participate in the study in accord with the rules of the process committee at the SNP Research Center, The Institute of Physical and Chemical Research (RIKEN).

Genotyping

To identify SNPs in the human *IRAK-M* and *SIGIRR* genes, we sequenced all exons, including a minimum of 200 bases of the flanking intronic sequence, 2 kb of the 5′ flanking region, and a 2 kb continuous 3′ flanking region of the last exon except for regions of interspersed repeats from 24 asthmatic subjects (12 unrelated children and 12 adults). Primer sets were designed on the basis of genomic sequences from the GenBank database (Table 1). The sequences were analyzed and polymorphisms identified using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI, USA). Genotyping of polymorphisms was performed by using the Invader assay or the TaqMan allele-specific amplification (TaqMan-ASA) method or PCR restriction fragment length polymorphism (PCR-RFLP) analysis as described (Hasegawa et al. 2004; Kamada et al. 2004). For the −1464A > G, 21927A > T, 22149G > A, 48837A > G, and 54406C > T polymorphisms in *IRAK-M*, genotyping was performed by the Invader method (Ohnishi et al. 2001). For the −1195C > T and 39384A > del polymorphisms in *IRAK-M* and the −10137C > T, −8778C > T, and 1523T > G polymorphisms in *SIGIRR*, genotyping was performed by the TaqMan method.

Statistical analysis

We calculated allele frequencies and tested agreement with Hardy–Weinberg equilibrium using a χ^2 goodness-of-fit test at each locus. To test the association between each gene and childhood or adult asthma, we compared differences in allele frequency and genotype distribution of each polymorphism between case and control subjects by using a contingency chi-square test with one degree of freedom (DF). Odds ratios (ORs) with 95 percent confidence intervals (95% CI) were also calculated.

In the association study between a single SNP and an asthma-related phenotype, we performed many statistical tests; therefore, inflation of the false-positive results (type-1 error) is a concern. In this study, we consider those results to be hypothesis-generating, and only results with P values of less than 0.01 are shown here to minimize type-1 errors.

Pairwise LD was calculated as $|D'|$ and r^2 by using the SNP Alyze statistical package (Dynacom, Chiba, Japan) as described by Nakajima et al. (2002). Haplotype frequencies for multiple loci were estimated using the expectation-maximization method with SNP Alyze software (Nakajima et al. 2002). Those frequencies in cases and controls were evaluated both by the whole

Table 2 Polymorphisms in the *IRAK-M* and *SIGIRR* genes

Name	SNP[a]	Location	Nucleotide	Position[b]	Amino acid	Minor allele frequency (%)	JSNP ID IMS-JST	NCBI dbSNP
IRAK-M	SNP 1	5′ genome	T > C	−1494		14	168748	rs1732888
	SNP 2[a]	5′ genome	A > G	−1464		14	168749	rs1732887
	SNP 3[a]	5′ genome	C > T	−1195		30	168750	rs2701653
	SNP 4	Intron 1	T > A	14340		27		rs1185630
	SNP 5	Intron 2	C > T	14622		22		rs1882200
	SNP 6	Intron 2	C > T	14785		2		
	SNP 7	Intron 2	T > C	15608		19	040604	rs2289134
	SNP 8	Intron 3	C > T	20320		20		rs11465955
	SNP 9	Intron 3	T > A	20780		2		
	SNP 10	Intron 4	G > A	21076		2		
	SNP 11	Intron 4	A > del	21141		27		rs3830660
	SNP 12[a]	Intron 4	A > T	21927		21	046869	rs2293657
	SNP 13	Intron 4	G > C	22000		2		
	SNP 14[a]	Exon 5	G > A	22149	V147I	25	046868	rs1152888
	SNP 15	Intron 8	T > C	39263		2		
	SNP 16[a]	Intron 8	A > del	39384		48		rs10716217
	SNP 17[a]	Intron 8	A > G	48837		10	138735	rs3782347
	SNP 18[a]	Intron 8	C > T	54406		41	138737	rs3782348
	SNP 19	Intron 9	A > G	55583		2		
SIGIRR	SNP 1[a]	5′ genome	C > T	−10137		10		
	SNP 2[a]	5′ genome	C > T	−8778		27		
	SNP 3	Intron 1	G > A	−259		4		rs11246149
	SNP 4	Intron 2	T > C	60		29		rs4074794
	SNP 5	Intron 2	T > C	283		29		rs4076104
	SNP 6	Intron 2	C > T	785		2		
	SNP 7	Intron 3	G > A	1320		2		
	SNP 8[a]	Intron 3	T > G	1523		29		rs7396562
	SNP 9	Exon 5	C > G	1921	P115R	2		
	SNP 10	Exon 8	C > T	2921	P256P	2		
	SNP 11	Intron 8	C > G	3195		2		
	SNP 12	Intron 8	A > G	3260		29		rs10902159

Position 1 is the A of the initiation codon
[a]SNPs were genotyped in this study
[b]Numbering according to the genomic sequence of *IRAK-M* (NT_029419.10) and *SIGIRR* (AC138230.5)

distribution with Fisher's exact test and by χ^2 tests of one haplotype against others (haplotype-wise test).

Results

Polymorphisms in the *IRAK-M* and *SIGIRR* genes

We performed screening of polymorphisms with genomic DNA from 24 randomly selected asthmatic individuals. After extensive examination of *IRAK-M* and *SIGIRR* by direct sequencing, we identified 19 polymorphisms in *IRAK-M* and 12 SNPs *in SIGIRR* (Table 2). Eighteen polymorphisms were contained in the two available public databases; NCBI dbSNP (http://www.ncbi.nlm nih.gov/SNP/) and IMS-JST JSNP DATABASE (http://www.snp.ims.u-tokyo.ac.jp/). Non-synonymous substitutions were located in *IRAK-M* (Val147Ile) and *SIGIRR* (Pro115Arg). IRAK molecules consist of two major functional domains, death domain and kinase domain. SNP14 V147I did not locate in these functional domains. SIGIRR contains the immunoglobulin (Ig) domain and Toll and interleukin-1 receptor (TIR) domain, and the SNP9 P115R located in the Ig domain. To examine the LD between identified SNPs, pairwise LD coefficients D' and r^2 were calculated using the SNP Alyze program. Because most of the SNPs were quite rare, pairwise LD was measured by $|D'|$?and r^2 among the SNPs with a frequency of greater than 5%. Results for the molecules are shown in Tables 3 and 4. The LD pattern in four different ethnic populations is available on the website http://www.hapmap.org. The LD pattern using HapMap data of *IRAK-M* SNPs identified in this study is shown in a table in the supplementary material. The LD pattern in the Japanese was significantly different from that in the Yoruba, and almost the same as that in the Chinese. In the *SIGIRR* gene, SNPs identified in this study are not contained in HapMap database. In *IRAK-M* gene, SNP1 was in complete LD ($D' = 1.00$ and $r^2 = 1.00$) with SNP2. SNP12 was in complete LD with SNP4 and SNP5, and was in strong LD ($D' = 1.00$ and $r^2 = 0.87$) with SNP7 and SNP8. In the *SIGIRR* gene, SNP8 was in complete LD with SNP4, SNP5, and SNP11. We finally selected ten polymorphisms for association studies. In addition, we searched the putative transcription factor binding site using TFSEARCH (http://www.mbs.cbrc.jp/research/db/TFSEARCH.html) (Heinemeyer et al. 1998). We found that SNP3 AAACAA(C > T) in the *IRAK-M* gene contains putative SRY binding site with higher probability (96.4 vs. 90.0%, respectively).

Table 3 Pairwise linkage disequilibrium for all possible two-way comparisons among 13 SNPs in *IRAK-M*

	SNP 1	SNP 2	SNP 3	SNP 4	SNP 5	SNP 7	SNP 8	SNP 11	SNP 12	SNP 14	SNP 16	SNP 17	SNP 18
SNP 1	*D*'	1.00	1.00	1.00	0.74	0.04	0.47	1.00	0.74	1.00	1.00	0.02	1.00
	r^2	1.00	0.07	0.07	0.02	0.00	0.01	0.06	0.02	0.06	0.14	0.00	0.09
SNP 2		*D*′	1.00	1.00	0.74	0.04	0.47	1.00	0.74	1.00	1.00	0.02	1.00
		r^2	0.07	0.07	0.02	0.00	0.01	0.06	0.02	0.06	0.14	0.00	0.09
SNP 3			*D*′	0.87	1.00	1.00	1.00	0.87	1.00	0.87	0.64	1.00	0.54
			r^2	0.68	0.11	0.11	0.10	0.60	0.11	0.61	0.20	0.05	0.20
SNP 4				*D*′	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.48
				r^2	0.08	0.08	0.10	1.00	0.08	1.00	0.45	0.03	0.15
SNP 5					*D*′	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.15
					r^2	0.86	0.86	0.11	1.00	0.10	0.30	0.39	0.01
SNP 7						*D*′	1.00	1.00	1.00	1.00	1.00	1.00	0.10
						r^2	0.71	0.11	0.86	0.09	0.24	0.45	0.00
SNP 8							*D*′	1.00	1.00	1.00	1.00	1.00	0.23
							r^2	0.09	0.87	0.09	0.29	0.32	0.02
SNP 11								*D*′	1.00	1.00	1.00	1.00	0.47
								r^2	0.10	1.00	0.38	0.05	0.13
SNP 12									*D*′	1.00	1.00	1.00	0.14
									r^2	0.09	0.29	0.39	0.01
SNP 14										*D*′	1.00	1.00	0.45
										r^2	0.36	0.04	0.11
SNP 16											*D*′	1.00	0.57
											r^2	0.11	0.23
SNP 17												*D*′	1.00
												r^2	0.07

Association of each SNP with asthma and asthma related-phenotypes

Ten SNPs were genotyped in 391 patients with childhood asthma, 462 patients with adult asthma, and 639 controls. All genotype results of the SNPs in the control samples were in Hardy–Weinberg equilibrium. The results of allele frequencies in the asthmatic and control groups are shown in Table 5. None of the SNPs tested in this study had significant association with adult or childhood asthma.

In addition, we surveyed associations between SNPs of those two genes and asthmatic patients with a high eosinophil count, a high serum IgE level, disease severity, atopic asthma, and child asthmatic patients with atopic dermatitis. There was no association between any SNP of *IRAK-M* and *SIGIRR* genes and asthma-related phenotype.

Power in this study was estimated with the aid of SamplePower 2.0 (SPSS, Chicago, IL, USA). If ORs of risk alleles with control group frequencies of 0.05, 0.1, 0.2, and 0.4 were more than 1.86, 1.60, 1.44, and 1.37, respectively, power exceeded 80% (at $P = 0.01$) in allelic association tests of childhood asthma (639 controls and 391 patients). Similarly, in allelic association tests in adult asthma (639 controls and 462 patients), power of 80% was assured if alleles with frequencies of 0.05, 0.1, 0.2, and 0.4 had ORs of more than 1.81, 1.56, 1.42, and 1.35, respectively.

Association between haplotypes of the *IRAK-M* and *SIGIRR* genes and asthma

We next constructed the haplotypes of those three genes and estimated the frequency of each haplotype in the control, childhood asthma, and adult asthma groups (Table 6). The frequency pattern of the haplotypes of *IRAK-M* and *SIGIRR* did not differ between the control and asthma groups.

Table 4 Pairwise linkage disequilibrium for all possible two-way comparisons among six SNPs in *SIGIRR*

	SNP 1	SNP 2	SNP 4	SNP5	SNP 8	SNP 12
SNP 1	*D*′	1.00	1.00	1.00	1.00	1.00
	r^2	0.04	0.05	0.05	0.05	0.05
SNP 2		*D*′	0.77	0.77	0.77	0.77
		r^2	0.53	0.53	0.53	0.53
SNP 4			*D*′	1.00	1.00	1.00
			r^2	1.00	1.00	1.00
SNP 5				*D*′	1.00	1.00
				r^2	1.00	1.00
SNP 8					*D*′	1.00
					r^2	1.00

Table 5 Genotype frequencies for *IRAK-M* and *SIGIRR* SNPs and asthma susceptibility

Gene	SNP location	Control ($n=639$)			Child BA ($n=391$)			Adult BA ($n=462$)			Child BA			Adult BA		
		1	2	3	1	2	3	1	2	3	P^a	P^b	P^c	P^a	P^b	P^c
IRAK-M	SNP 2	0.81	0.18	0.01	0.82	0.17	0.01	0.80	0.19	0.01	NS	NS	NS	NS	NS	NS
	SNP 3	0.33	0.51	0.16	0.30	0.55	0.15	0.30	0.50	0.20	NS	NS	NS	NS	NS	NS
	SNP 12	0.76	0.22	0.02	0.72	0.26	0.02	0.78	0.21	0.01	NS	NS	NS	NS	NS	NS
	SNP 14	0.35	0.50	0.15	0.33	0.52	0.14	0.33	0.48	0.19	NS	NS	NS	NS	NS	NS
	SNP 16	0.30	0.48	0.21	0.31	0.49	0.20	0.32	0.46	0.22	NS	NS	NS	NS	NS	NS
	SNP 17	0.92	0.07	0.00	0.93	0.07	0.00	0.93	0.07	0.00	NS	NS	NS	NS	NS	NS
	SNP 18	0.38	0.46	0.16	0.35	0.51	0.15	0.36	0.48	0.16	NS	NS	NS	NS	NS	NS
SIGIRR	SNP 1	0.86	0.13	0.01	0.86	0.13	0.01	0.85	0.14	0.00	NS	NS	NS	NS	NS	NS
	SNP 2	0.61	0.35	0.05	0.59	0.35	0.05	0.60	0.34	0.05	NS	NS	NS	NS	NS	NS
	SNP 8	0.44	0.44	0.12	0.45	0.44	0.11	0.44	0.45	0.11	NS	NS	NS	NS	NS	NS

NS Not significant
[a]Dominant model
[b]Recessive model
[c]Allele frequency

Discussion

Recent studies have shown that the immune response induced by an endotoxin could play an important role in the initiation or prevention of asthma (Braun-Fahrlander et al. 2002; Gereda et al. 2000; Gehring et al. 2002). Immunization with an antigen in the context of TLR2 ligands can result in experimental asthma (Redecke et al. 2004) and genetic variation in *TLR2* is a major factor in the susceptibility to asthma of children of farmers (Eder et al. 2004). Although no association was observed between *TLR4* polymorphism and the risk of asthma (Raby et al. 2002), several reports have shown that *TLR4* gene variants modify endotoxin effects on asthma and relate to the severity of asthma (Yang et al. 2004). Given these studies, the TLR signaling pathway seems to be a possible inducer of the immune deviation that affects asthma susceptibility. We identified polymorphisms in *IRAK-M* and *SIGIRR*, and performed case-control and case-only association studies and haplotype analyses using clinically characterized asthma patients. In this study, no significant association between the tested SNPs in *IRAK-M* or *SIGIRR* and asthma or any asthma-related phenotype was found. In this study, if the allelic OR was more than 1.86 with a risk allele frequency of 0.05 in the control group, power exceeded 80% in association tests of childhood asthma. Similarly, power of 80% was assured in association tests of adult asthma if the allelic OR was greater than 1.81 with a control group risk allele frequency of 0.05. It is possible that rare variants are associated with the development of asthma. We screened a minimum of 200 bases of the flanking intronic sequence, 2 kb of the 5′ flanking region, and a 2 kb continuous 3′ flanking region to the last exon, although other SNPs in unsequenced regions might be associated with asthma or its related phenotypes. On the other hand, a gene–environment interaction might affect these results. Recent studies have shown that exposure to germs early in life

Table 6 Haplotype frequencies of polymorphisms of the *IRAK-M* and *SIGIRR* genes

	Haplotype no.	SNP position							Child BA ($n=391$)	Adult BA ($n=462$)	Controls ($n=639$)
IRAK-M		SNP 1	SNP 3	SNP 12	SNP 14	SNP 16	SNP 17	SNP 18			
	1	A	C	A	G	del	A	C	0.31	0.30	0.32
	2	A	T	A	A	A	A	T	0.30	0.30	0.30
	3	G	C	A	G	del	A	C	0.07	0.09	0.09
	4	A	T	A	A	A	A	C	0.09	0.10	0.08
	5	A	C	T	G	A	A	T	0.06	0.05	0.06
	6	A	C	T	G	A	G	C	0.03	0.03	0.04
	7	A	C	T	G	A	A	C	0.05	0.03	0.03
	8	A	T	A	G	del	A	C	0.03	0.03	0.02
	9	A	C	A	G	del	A	T	0.02	0.02	0.01
	Others								0.04	0.05	0.04
SIGIRR	Haplotype no.	SNP 1	SNP 2	SNP 8							
	1	C	C	T					0.59	0.58	0.57
	2	C	T	G					0.17	0.17	0.17
	3	C	C	G					0.12	0.13	0.13
	4	C	T	T					0.05	0.04	0.05
	5	T	C	T					0.04	0.03	0.04
	Others								0.03	0.04	0.03

may facilitate the development of an immune system that is appropriately balanced with respect to Th1 and Th2 cells (Braun-Fahrlander et al. 2002; Gereda et al. 2000; Gehring et al. 2002). TLRs contact the environment, and play a crucial role in host defense against infection (Medzhitov 2001; Akira and Takeda 2004). Subjects carrying wild-type *TLR4* genotypes have an increased risk of asthma with greater endotoxin exposure but there is no such effect in subjects with variant genotypes (Werner et al. 2003). Eder et al. (2004) showed that the *TLR2* gene is a major factor in the susceptibility of children of European farmers to asthma. We recruited subjects from the Osaka area, an urban area in Japan. A genetic variation in *IRAK-M* and *SIGIRR* might be one determinant of susceptibility to asthma in a farming environment. In addition, epistatic interactions may affect the results.

Innate immunity plays a major role in host defense during the early stages of infection, and differences in population history have produced unique patterns of SNP allele frequencies, LD, and haplotypes when ethnic groups are compared (Lazarus et al. 2002). Analysis of genetic variation in 16 innate immunity genes of African Americans, European Americans, Hispanic Americans, and Asthmatic Europeans has revealed higher haplotype diversity among the African Americans (Lazarus et al. 2002). In the *IRAK-M* gene, the LD pattern in Japanese was significantly different from that in Yoruba and was almost the same as that in Chinese.

The function of TLRs in various human diseases has been investigated, and these studies have shown that TLR function affects several diseases such as sepsis, immunodeficiencies, and atherosclerosis (Cook et al. 2004). Mice deficient in SIGIRR have a very similar phenotype to that of IRAK-M-deficient mice in terms of LPS hyperresponsiveness (Wald et al. 2003). It is possible that *SIGIRR* or *IRAK-M* polymorphisms contribute to the etiology of other diseases, for example bacterial infections. In this, we newly identified a non-synonymous substitution in *SIGIRR* (Pro115Arg). The variants, *IRAK-M* (Val147Ile) and *SIGIRR* (Pro115Arg), might be associated with the etiology of another disease, by alteration of protein function.

In this study we found that the region containing SNP3 in the *IRAK-M* gene is more likely to contain a putative SRY-binding site. The sex-determining region on the Y chromosome (*SRY*) is a master gene that initiates testis differentiation in mammals. *SRY* and *SOX* (for "*SRY*-like HMG-box-containing") belong to the same family, which contain an "HMG box", a protein domain that binds to DNA at a target sequence (Marshall Graves 2002). In previous studies, Sox-4 seemed crucial for B-lymphopoiesis and thymocyte development (Smith and Sigvardsson 2004), but the relationship between the transcription factor SRY and development of immune cells remained unclear.

Although we could not find any significant association between the tested polymorphisms and asthma susceptibility or asthma-related phenotype, our findings will be helpful for choosing SNPs for further association and functional studies of other diseases. Examinations on other molecules in the TLR signaling pathway are needed to clarify the pathogenesis of asthma.

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