

Significant Evidence for Linkage of Mite-Sensitive Childhood Asthma to Chromosome 5q31–q33 near the Interleukin 12 B Locus by a Genome-wide Search in Japanese Families

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Childhood-onset asthma is frequently found in association with atopy. Although asthmatic children may develop IgE antibodies against variety of allergens, asthma is associated primarily with allergy to house-dust mites, molds, or other allergens. In this study, we conducted a genome-wide linkage search in 47 Japanese families (197 members) with more than two mite-sensitive atopic asthmatics (65 affected sib-pairs) using 398 markers. Multipoint linkage analysis was carried out for atopic asthma as a qualitative trait using the MAPMAKER/SIB program. We observed significant evidence for linkage with maximum lod scores (MLS) of 4.8 near the interleukin 12 B gene locus on chromosome 5q31–q33. In addition, suggestive evidence on 4q35 with MLS = 2.7 and on 13q11 with MLS = 2.4 was obtained. The other possible linkage regions included 6p22–p21.3 (MLS = 2.1), 12q21–q23 (MLS = 1.9), and 13q14.1–q14.3 (MLS = 2.0). Many of the linkage loci suggested in this study were at or close to those suggested by genome-wide studies for asthma in Caucasian populations. The present study suggests the contribution of the interleukin 12 B gene or nearby gene(s) to mite-sensitive atopic asthma and a considerable number of genetic variants common across Caucasians and Japanese populations contributing to asthma, although the relative importance of various variants may differ between the groups. © 2000

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INTRODUCTION

Asthma is one of the most common chronic diseases of childhood in developed countries. Susceptibility to

asthma is related to the interaction of multiple genes and environmental factors. Family and twin studies have suggested the presence of major genes controlling susceptibility to atopy (Borecki *et al.*, 1985; Dizier *et al.*, 1995). Several loci possibly linked to atopy and/or asthma have been suggested through genome-wide linkage studies involving a British/Australian sample of atopic sib-pairs (Daniels *et al.*, 1996); different American populations including African-American, Hispanic, and Caucasian samples of asthmatic sib-pairs and extended families (CSGA, 1997; Hizawa *et al.*, 1998a,b,c); a founder population in Hutterites living in South Dakota (Ober *et al.*, 1998); and German and Swedish families of asthmatic sib-pairs (Wjst *et al.*, 1999). Several chromosome regions of candidate genes for atopy and asthma have also been investigated for linkage and association (Barnes *et al.*, 1996; Caraballo and Hernandez, 1990; Cookson *et al.*, 1989; Hizawa *et al.*, 1998b; Marsh *et al.*, 1994; Moffatt *et al.*, 1994; Noguchi *et al.*, 1998). However, the results of these studies were considerably inconsistent, probably due to different populations and/or to different atopic phenotypes in addition to false-positive and false-negative results. If a reported linkage is true, linkage results are expected to be replicated when investigated using the same phenotypic definition in the same ethnic population. Since it is suggested that susceptibility alleles at loci influencing asthma phenotypes are likely to be common polymorphisms in the population (Ober *et al.*, 1998), they are likely to be shared across several human populations (Halushka *et al.*, 1999). Therefore, shared findings across different populations are likely to specify true linkage loci.

Childhood-onset asthma is frequently found with the presence of atopy, which is the genetic susceptibility to produce IgE directed toward common environmental allergens, including house-dust mites, animal proteins, and fungi (Larsen, 1992). Although asthmatic children

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TABLE 1
Characteristics of the Families

No. of families	47
No. of families with no asthma affected parent	34
No. of families with one asthma affected parent	12
No. of families with two asthma affected parents	1
No. of families with no mite-sensitive parent	20
No. of families with one mite-sensitive parent	18
No. of families with two mite-sensitive parents	9
No. of families with no mite-sensitive asthma parent	40
No. of families with one mite-sensitive asthma parent	6
No. of families with two mite-sensitive asthma parents	1
Affected children	
Number	102
Mean $\pm$ SD age (years)	11.2 $\pm$ 6.2
Sex ratio (M/F)	58/36
Mean total IgE $\pm$ SD (IU/ml)	1340.8 $\pm$ 1626.5
No. with total IgE $>$ +1 SD above age-matched population mean	102
No. with total IgE $>$ +2 SD above age-matched population mean	80
Mean Df RAST IgE $\pm$ SD (IU/ml)	67.3 $\pm$ 35.3
Parents	
Number	94
Mean $\pm$ SD age (years)	41.1 $\pm$ 7.8
Mean total IgE $\pm$ SD (IU/ml)	336.6 $\pm$ 563.6
No. with total IgE $>$ +1 SD above age-matched population mean	40
No. with total IgE $>$ +2 SD above age-matched population mean	26
Mean Df RAST IgE $\pm$ SD (IU/ml)	11.42 $\pm$ 23.38

Note. Df: *Dermatophagoides farinae*.

may develop IgE antibodies against a variety of allergens, asthma and bronchial hyperresponsiveness are associated primarily with allergy to house-dust mites or molds and to a lesser degree to cat dander and grasses (Cookson *et al.*, 1991; Sears *et al.*, 1989). In Japan, more than 85% of children with asthma have IgE antibodies against the house-dust mite, *Dermatophagoides farinae* (Df) (Shibasaki *et al.*, 1988). In this study, to ensure phenotypic homogeneity, we have selected mite-sensitive atopic asthmatics for our affected sib-pair analysis and conducted a genome-wide linkage analysis. This is the first report of a genome-wide search for asthma carried out in an Asian population.

SUBJECTS AND METHODS

Subjects and families. Probands of the families studied were asthmatic children who visited the Pediatric Allergy Clinic of the University Hospital of Tsukuba. A full verbal and written explanation of the study was given to all family members interviewed, and 86 families (375 members including 172 atopic asthmatic children) gave us informed consent and participated in this study. For subjects younger than 5 years of age, informed consent was given by their parents. The mean age of the probands and their siblings was 11.2 years (range: 3 to 29 years); the mean age of the parents was 41.1 years (range: 28 to 72 years). Each family member was questioned regarding allergic symptoms and underwent a physical examination by participating physicians or pediatricians. Asthma was diagnosed in subjects according to the criteria of the National Institutes of Health, (Bethesda, MD), with minor modifications (National Heart, Lung, and Blood Institute, National Institutes of Health, 1997). Patients had to show the two following characteristics: (1) two or more episodes of wheezing and shortness of breath during the past year and (2) reversibility of the wheezing and dyspnea, either spon-

taneously or by bronchodilator treatment. Though methacholine challenge was not performed because of the young ages of the affected sib-pairs, the diagnosis of asthma in this population was confirmed clinically by participating physicians or pediatricians who had treated the children for longer than 2 years. Since wheezing is often associated with viral respiratory infection in young children, we excluded children younger than 3 years old. The young adult patients included in this study had suffered from chronic asthma since childhood. Subjects with any other complicating lung diseases, cigarette exposure history, or birth weight under 2000 g were excluded. Total serum IgE levels and specific IgE levels to Df were determined with the Pharmacia CAP System (Uppsala, Sweden). To achieve homogeneity of the asthmatic patients, we selected families having more than two asthmatic children with a Df RAST score $>$ 3. Of 102 members of 65 mite-sensitive asthmatic sib-pairs, 80 members including 46 sib-pairs had a total serum IgE level greater than 2 standard deviations above the geometric mean for the age-matched normal Japanese population, and the remaining 22 members of 19 sib-pairs had between 1 and 2 standard deviations above the age-matched geometric mean. These childhood-onset, mite-sensitive atopic asthma-affected sib-pairs and their parents were subjected to genotyping (Table 1: 47 families, 196 members, and 65 affected sib-pairs). The numbers of affected children per family were 2 in 41 families, 3 in 4 families, and 4 in 2 families. The affected sibs included 58 males and 36 females. The number of families with none, one, and two affected parents with asthma was 33, 13, and 1, respectively. There were nonpaternities in none of the families. An absence of nonpaternity was ensured by no inconsistent allelic transmission of 391 microsatellite markers in the families. Because the age of onset may vary between individuals, additional possibly unaffected siblings were not analyzed to avoid the incorrect definition of unaffectedness. The families examined in this study are the same families that participated in our previous linkage and association study on chromosome 13 (Kimura *et al.*, 1999). Some of the subjects of this study (19 families and 26 affected sib-pairs) also participated in our previous linkage study on chromosome regions 5q31–q33 and 11q13

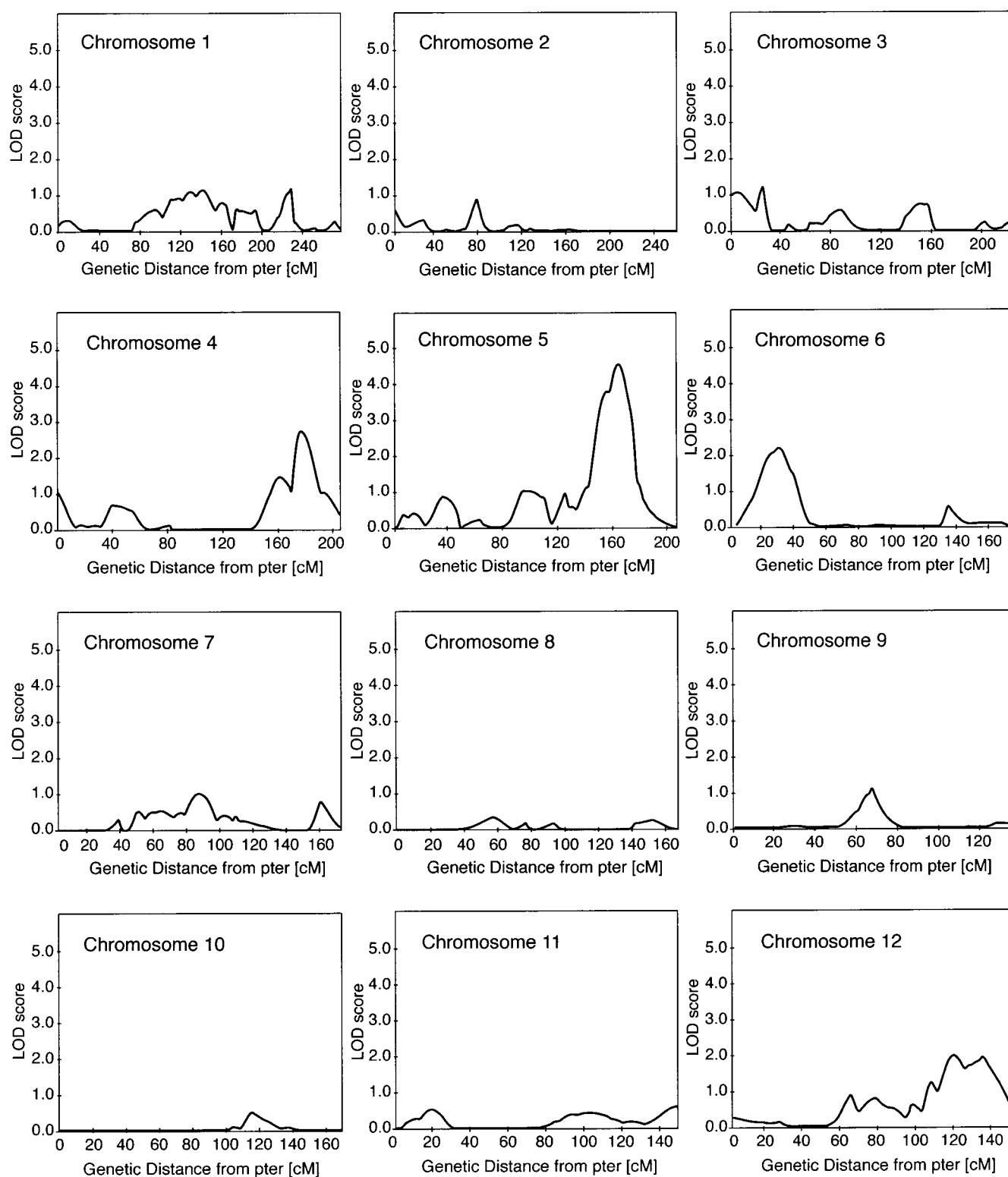


FIG. 1. Genome-wide scan for mite-sensitive atopic asthma in the Japanese population. A panel of 391 polymorphism markers was used. Each box represents a chromosome. The y-axis depicts the multipoint nonparametric maximum lod score. The x-axis represents the centimorgan distance of the markers used. The telomere is to the right.

(Noguchi *et al.*, 1997). The present study was approved by the Committee of Ethics of the University of Tsukuba.

Genotyping. DNA was extracted from peripheral blood leukocytes. Genotyping was performed using the Research Genetics version 8 linkage screening set (376 markers) (Research Genetics, Inc.,

Huntsville, AL; <ftp://ftp.resgen.com/pub/mappairs/humanset/>) except for 15 markers on chromosome 13, which are described elsewhere (Kimura *et al.*, 1999). For denser marker mapping for chromosome 5q31–q33, 7 additional markers were used: D5S2847, D5S2013, D5S2077, D5S1507, D5S1352, D5S422, and D5S2040. The

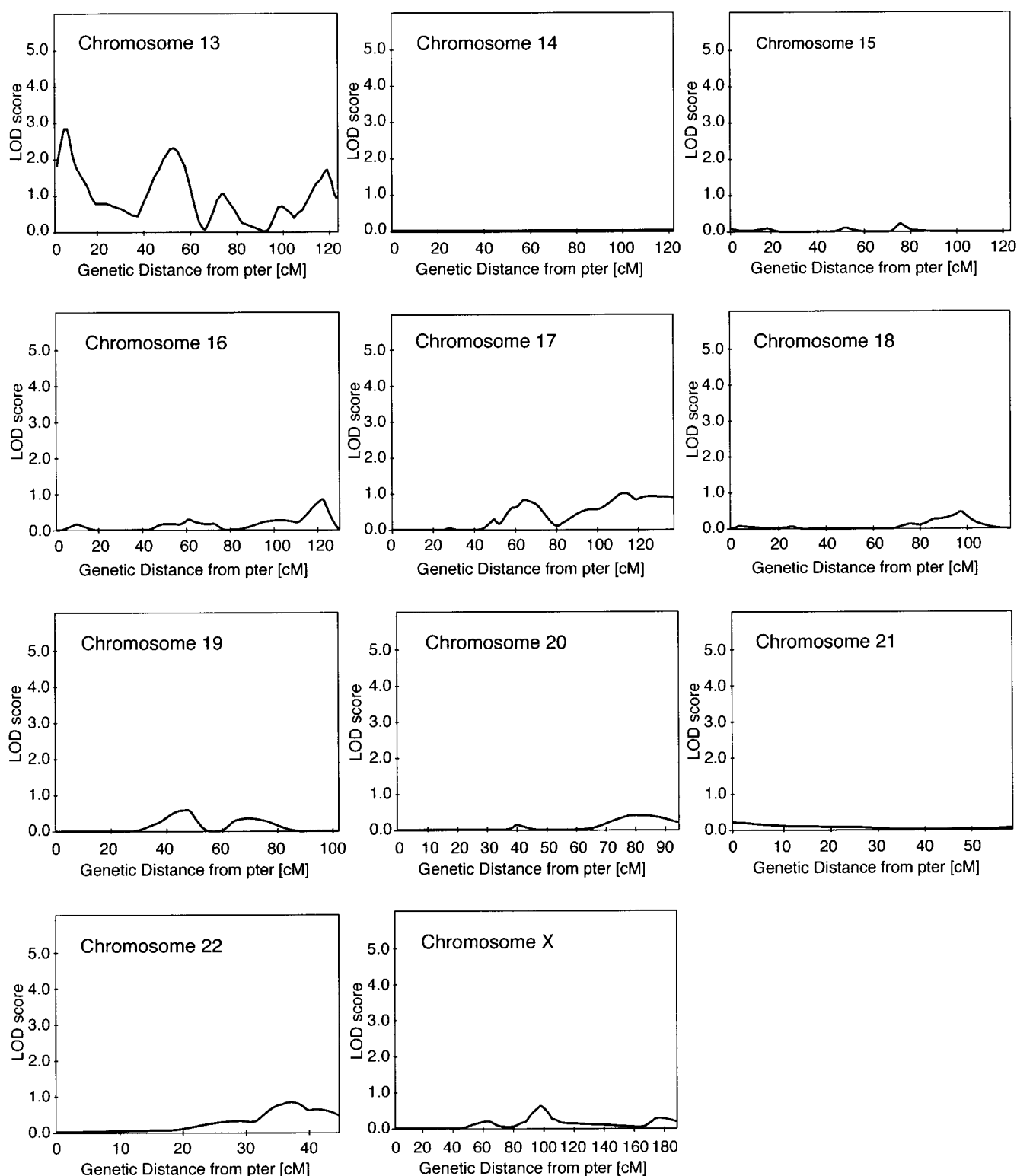
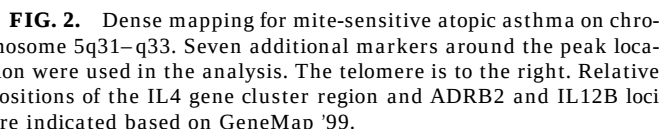


FIG. 1—Continued

additional markers were selected from the publicly available chromosome 5 genetic map at the Marshfield Medical clinic Web site (http://www.marshmed.org/genetics/Map_Markers/maps/index-map.html). The microsatellite markers were amplified by PCR with a primer pair for each microsatellite marker as follows. The PCR cocktail contained 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 1.0–1.5 mM MgCl₂; 0.1% Triton X-100; 200 μ M each of deoxynucleotide triphosphates (dNTPs); 0.6 U AmpliTaq DNA polymerase (Perkin-Elmer

Corp., Norwalk, CT); 5 μ M each of primers; and 20 ng of template DNA in a total volume of 10 μ l. PCR was performed in a 96-well plate with a programmable thermal cycler (GeneAmp PCR system 9600; Perkin-Elmer Corp.). The amplification was accomplished with a 2-min primer cycle at 95°C, followed by 30 cycles consisting of denaturation at 94°C for 45 s, annealing at 52 to 65°C for 45 s, and extension at 72°C for 60 s; followed by a final 7-min cycle at 72°C. Reactions for each marker were performed separately with the prod-



Linkage analysis. Allele frequencies were estimated from the parental chromosomes by the program SIB-PAIR (<http://www.qimr.edu.au/davidD/davidd.html>). Information about marker order and position was obtained from the Marshfield Medical Clinic Web site. Multipoint nonparametric linkage analysis was performed using the computer program MAPMAKER/SIBS version 2 (Kruglyak and Lander, 1995), which calculates the MLS at each point in the genome by estimating the maximum-likelihood sharing probabilities (IBD) for each sib-pair.

RESULTS

achieved the significant linkage level with a MLS of 4.52. Two regions, chromosome 4q35 and chromosome 13q11, achieved the suggestive linkage level with MLSs of 2.74 and 2.82, respectively. In addition, 15 regions showed an MLS > 1 . These regions were on chromosomes 1q, 3p, 3q, 4q, 6p, 7q, 9q, 12q, 13q, and 17q. An additional 7 markers were added to the 5q31–q33 region between D5S1480 and D5S1471 (Fig. 2). The MLS curve displayed two peaks with an MLS of 4.78 at marker D5S820 and an MLS of 3.73 at D5S1471, which is 12.3 cM telomeric to D5S820.

Genome-wide linkage screens for asthma/atopy have been conducted in Caucasians, Hispanics, African Americans, and, now, Japanese. Previous studies identified regions at 5q15 and 17p11.1-q11.2 in African Americans; at 5q23-q31, 6p21.3-p23, 11p15, 12q14-q24.2, 13q21.3-qter, and 19q13 in Caucasians; and at 2q33 and 12q14-q24.2 in Hispanics with suggestive evidence for linkage to asthma/atopy (CSGA, 1997). A recent genome-wide study conducted by Wjst *et al.* (1999) showed that regions at 2qter, 6p21.3, 9pter-qter, and 12q13 could be linked to asthma. Other regions are also noted for possible linkage to asthma/atopy (Daniels *et al.*, 1996; Ober *et al.*, 1998; Wjst *et al.*, 1999). In addition to these genome-wide linkage screens, many studies reported possible linkage regions. The present study identified one region (5q31-q33) with significant evidence and two regions (4q35 and 13q11) with suggestive evidence for linkage. Additionally, 15 possible linkage loci with MLS > 1 were identified (Table 2). Some of these loci with weak evidence for linkage are probably false-positive results. However, among these loci, chromosome regions 1q, 4q, 6p, 12q, 13q, and 17q were close to loci where linkage was suggested in Caucasian populations by genome-wide studies (Table 2), indicating the possibility that these loci include true linkage loci for asthma.

The interleukin 12B (IL12B) gene is mapped to

TABLE 2

Multipoint Linkage Results for Mite-Sensitive Atopic Asthma in the Present Study and Corresponding Findings of Linkage Studies for Asthma in Caucasian Populations

Present study				Studies in Caucasian populations			
Marker	Chromosome (position ^a)	Lod score	(<i>P</i> value)	Authors	Marker	Position ^a	<i>P</i> value
D1S1588–D1S1631	1 (125.5–136.8)	1.06	(0.01)	Ober <i>et al.</i> (1998)	D1S495	136.8	0.01
D1S1631–D1S534	1 (136.8–151.9)	1.10	(0.01)	Ober <i>et al.</i> (1998)	D1S221	142.24	0.03
D1S2141	1 (233.4)	1.10	(0.01)	Ober <i>et al.</i> (1998)	D1S549	239.7	0.009
D3S2387–D3S1304	3 (5.5–22.3)	1.02	(0.02)				
D3S4545–D3S2159	3 (26.3–36.6)	1.17	(0.01)				
D4S2368	4 (167.6)	1.45	(0.005)	Ober <i>et al.</i> (1998)	D4S2368	167.6	0.01
D4S2417–D4S408	4 (181.9–195.1)	2.74	(0.0002)	Wjst <i>et al.</i> (1999)	D4S1535	195.1	0.04
				Ober <i>et al.</i> (1998)	D4S408	195.1	0.045
D5S820	5 (159.8)	4.73	(1.3 × 10⁻⁶)	Ober <i>et al.</i> (1998)	D5S820	159.8	0.02
D5S1471	5 (172.1)	3.73	(1.7 × 10⁻⁵)	Ober <i>et al.</i> (1998)	D5S1456	174.8	0.03
D6S1959–D6S2439	6 (34.2–42.3)	2.13	(0.0009)	Wjst <i>et al.</i> (1999)	D6S422	35.6	0.015
				Ober <i>et al.</i> (1998)	D6S1281	44.4	0.007
				Wjst <i>et al.</i> (1999)	D6S276	44.4	0.02
D7S2204	7 (91.0)	1.01	(0.02)				
D9S301	9 (66.3)	1.05	(0.01)				
D12S346–D12S78	12 (104.7–111.9)	1.18	(0.01)	Wjst <i>et al.</i> (1999)	D12S327	97.78	0.02
				Barns <i>et al.</i> (1996)	D12S360	111.9	0.02
D12S78–D12S79	12 (111.9–125.3)	1.92	(0.001)				
D12S86	12 (134.5)	1.87	(0.002)	Wilkinson <i>et al.</i> (1998)	D12S366	133.3	
D13S175–D13S217	13 (6.0–17.2)	2.41	(0.0004)	Ober <i>et al.</i> (1998)	D13S787	8.87	0.0025
D13S153	13 (45.6)	2.02	(0.001)				
D13S265	13 (68.7)	1.41	(0.005)				
D17S784	17 (116.9)	1.12	(0.01)	Ober <i>et al.</i> (1998)	D17S784	116.9	0.02

^a Genetic distance was given from the p-terminal (cM) based on the Marshfield sex-averaged genetic maps. Boldface type indicates the markers showing significant and suggestive evidence for linkage to atopic asthma.

5q31–q33 (Sieburth *et al.*, 1992; Warrington and Bengtsson, 1994) within 5 cM telomeric to D5S820 and near D5S1352 (GeneMap'99 and the genetic maps at the Marshfield Medical Clinic Web site). An MLS of 4.34 to D5S1352 was reported in this study. IL12 is a key cytokine for the induction of Th1-type immune responses, and a reduced capacity to produce this cytokine could lead to aberrant Th2-type responses in atopic patients, which is considered responsible for the class switch to IgE antibody formation (van der Pouw Kraan *et al.*, 1997). *In situ* hybridization showed a lower number of IL12 mRNA-positive cells in bronchial biopsies from asthmatic subjects than in those from normal control subjects (Naseer *et al.*, 1997). In a murine model of allergen-induced airway eosinophilia and bronchial hyperresponsiveness (BHR), IL12 can suppress antigen-induced airway changes despite the presence of circulating specific IgE (Kips *et al.*, 1995, 1996; Lee *et al.*, 1999). IL12 also inhibits the production of interleukin 5 by antigen-stimulated mononuclear cells, partially dependent on interferon- γ production, and inhibits the release of eosinophil survival-stimulating factor from mononuclear cells (Okano *et al.*, 1998). Therefore, it is possible that the IL12B gene may play some role in the development of atopic asthma.

Our previous study suggested a linkage of asthma to the IL4 gene cluster region at 5q31–q33 ($P = 0.0013$ at IL4 and $P = 0.0077$ at D5S393) (Noguchi *et al.*, 1997).

In the present study, MLS was 1.16 ($P = 0.01$) at D5S816, which is 0 cM from IL9. Since we selected mite-sensitive atopic asthma families in our current study, only 19 families overlapped between this study and our previous studies. Reanalysis by excluding the 19 families that were analyzed in our previous study resulted in an MLS of 0.86 ($P = 0.02$). Therefore, the linkage result at the IL4 gene cluster region in our new families in this study appears to be consistent with that of our previous study. However, it is possible that this weak linkage was influenced by the strong linkage to the IL12B region.

We observed an MLS of 3.73 at D5S1471. However, the MLS may be inflated by the linkage to D5S820 because D5S1471 is only from 12.3 cM from D5S820. Although we do not know how to evaluate the linkage to this region at present, possible linkage is suggested in the Hutterites at D5S156, 3 cM telomeric to D5S1471 (Asthma Gene Database, Ober *et al.*, 1998).

In our present study, suggestive evidence for linkage to atopic asthma was observed between D4S2417 and D4S408 on chromosome 4q33–q35. Possible linkage of asthma to D4S408 in the Hutterites ($P = 0.046$, Asthma Gene Database; Ober *et al.*, 1998) and to D4S1535, the same position to D4S408, in German/Swedish families ($P = 0.04$, Asthma Gene Database, Wjst *et al.*, 1999) was observed. One of the candidate genes located in this region is the gene encoding interferon regulatory factor 2 (IRF2), which is mapped to

TABLE 3

Reported Linkages with $P < 0.00073$ to Atopy-Associated Phenotypes and the Results of the Present Study

Reported studies					Present study		
Marker	Chromosome (position ^a)	Phenotype	P value	Authors	Marker	Position ^a	P value
DXS154	104.8	BHR, asthma	<0.0006	Holroyd <i>et al.</i> (1998)	DXS154	104.8	0.18
D4S1647	104.9	IgE	0.0005	Grimbacher <i>et al.</i> (1999)	D4S1647	104.9	0.99
D4S428	64.2	IgE	<0.0001	Grimbacher <i>et al.</i> (1999)	D4S1627	60.2	0.06
IL4	134.9–140.8	IgE	<0.0001	Marsh <i>et al.</i> (1994)	D5S816	139.3	0.02
D5S393	140.7	Atopy, IgE, SPT	0.0001	Noguchi <i>et al.</i> (1997)	D5S816	139.3	0.02
D5S500	140.7	Eosinophil cell	0.0006	Martinez <i>et al.</i> (1998)	D5S816	139.3	0.02
D5S436	147.5	IgE	0.0003	Meyers <i>et al.</i> (1994)	D5S1480	147.5	0.005
D5S436	147.5	IgE	0.0003	Bleecker <i>et al.</i> (1995)	D5S1480	147.5	0.005
D5S436	147.5	IgE	<0.0001	Xu <i>et al.</i> (1995)	D5S1480	147.5	0.005
D6S426	60.4	Eosinophil cell	0.0005	Wjst <i>et al.</i> (1999)	D6S1017	63.3	0.48
D7S684	147.2	IgE	0.0005	Noguchi <i>et al.</i> (1998)	D7S1824	149.9	0.34
FCER1B	62.5–65.0	IgE, asthma	<0.0001	Daniels <i>et al.</i> (1996)	D11S1765	65.0	0.42
D11S987	67.8	SPT	0.0005	Hizawa <i>et al.</i> (1998c)	D11S2371	76.2	0.47
D11S527	88.5	BHR	0.0003	Doull <i>et al.</i> (1996)	D11S2002	85.5	0.23

^a Genetic distance was given from the p-terminal (cM) based on the Marshfield sex-averaged genetic maps. BHR, bronchial hyperresponsiveness; SPT, skin prick test; eosinophil cell, eosinophil counts.

4q35.1 and located near D4S408 (GeneMap'99). The IRF1 gene, located on chromosome 5q31.1, is a transcriptional activator, which can positively regulate Th1-type response and negatively regulate Th2-type response (Taki *et al.*, 1997). IRF2 was identified as antagonistic repressor of IRF1 (Vaughan *et al.*, 1997; Taniguchi *et al.*, 1998). Furthermore, IRF2 itself is reported to be involved in the regulation of IL4 gene expression (Li-Weber *et al.*, 1994, 1995).

Our current findings include suggestive evidence for linkage to atopic asthma between D13S175 (13q11) and D13S217 on chromosome 13q12. Details of the data on chromosome 13 were described previously (Kimura *et al.*, 1999).

In this study, we analyzed only mite-sensitive childhood asthmatics to ensure phenotypic homogeneity. The subjects selected allowed us to use a simple non-parametric sibling pair analysis method for one phenotype. Although this approach can avoid nuisance problems concerning corrections for multiple testing of additional traits, results might be misleading in a complex disease where additional definitions are possible. Table 3 shows the markers where suggestive linkage (lod scores > 2.2; P value < 0.00074) to atopy-associated phenotypes including the hyper-IgE syndrome (<http://cooke.gsf.de/asthmagen/main.cfm>) was reported with our results of this study at the same markers or nearby markers. Except for DXS154 and FCER1B, reported linked phenotypes are not asthma. The present study did not provide supportive evidence for linkage to the regions listed in Table 3 other than chromosome 5q. However, given the different phenotypes and different ascertainment schemes, our negative findings are difficult to interpret.

In this genome-wide linkage study on mite-sensitive atopic asthma in a Japanese population, we noticed that four regions with MLS greater than 2 ($P < 0.001$),

4q, 5q, 6p, and 13q, are at or close to the regions suggested for linkage to asthma by genome-wide studies in Caucasian populations (Table 2). Although further effort is required to address whether favored linkage data arose from the same loci between studies, this apparent concordance indicates that the linkage findings of this study are not attributable to chance and that genetic variants contributing to asthma may show considerable similarities across Caucasians and Japanese populations although their relative importance may differ.

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