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A Second-Generation Genomewide Screen for Asthma-Susceptibility Alleles in a Founder Population

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A genomewide screen for asthma- and atopy-susceptibility loci was conducted, using 563 markers, in 693 Hutterites who are members of a single 15-generation pedigree, nearly doubling the sample size from the authors' earlier studies. The resulting increase in power led to the identification of 23 loci in 18 chromosomal regions showing evidence for linkage that is, in general, 10-fold more significant ($P < .001$ vs. $P < .01$) than the linkages reported previously in this population. Moreover, linkages to loci in 11 chromosomal regions were identified for the first time in the Hutterites in this report, including five regions (5p, 5q, 8p, 14q, and 16q) showing evidence both of linkage, by the likelihood ratio (LR) χ^2, and of disequilibrium, by the transmission/disequilibrium test. A region on chromosome 19 continues to show evidence for linkage, by both tests, in this study. Studies of 17 candidate genes provide evidence for association with variation in the *IL4RA* gene (16p12), the HLA class II genes (6p21), and the interferon-α gene cluster (9p22), but the lack of evidence for linkage in these regions by the LR χ^2 test suggests that these are minor susceptibility loci. A polymorphism in the *CD14* gene is in linkage disequilibrium with an as yet unidentified susceptibility allele in the 5q cytokine cluster, a region showing evidence for linkage among the Hutterites. Finally, 10 of the regions showing evidence for linkage in the Hutterites have shown evidence of linkage to related phenotypes in other genome screens, suggesting that these regions may contain common alleles that have relatively large effects on asthma and atopy phenotypes in diverse populations.

Introduction

The identification of susceptibility loci for common diseases, such as asthma, is complicated by genetic heterogeneity, reduced penetrance of the at-risk genotypes, and the confounding effects of environmental risk factors. Furthermore, susceptibility alleles for complex genetic diseases are likely to be common variants that show linkage disequilibrium over relatively short distances (Clark et al. 1998; Kruglyak 1999; Ober et al. 2000). Founder populations offer well-known advantages for the mapping of disease genes (Lander and Schork 1994; Wright et al. 1999), and relatively young populations that are derived from a small founding gene pool may be particularly useful for the mapping of genes for common, complex diseases (Kruglyak 1999).

We have focused our studies on the genetics of asthma in one such founder population, the Hutterites. The 693 Hutterites in our studies are related to one another in a 13-generation, 1,623-member pedigree (Abney et al. 2000*a*). All members of this pedigree can be traced back to 64 founders who lived during the early 1700s to early 1800s (Ober et al. 1997). The small number of founding genomes should minimize genetic heterogeneity, and the short time since their founding should maximize linkage disequilibrium, facilitating the search for asthma-susceptibility loci. In addition to the features common to all founder populations, the Hutterites practice a communal, agrarian lifestyle. This results in relatively homogeneous environments both within and between communal farms, which should minimize the confounding effects of environmental risk factors and, perhaps, enhance the effects of individual susceptibility loci. Of particular relevance to our studies of asthma is that smoking is prohibited and that, as a result, the Hutterites experience no first- or second-hand exposure to cigarette smoke.

The results of a genome screen using ~300 microsatellite markers for asthma- and atopy-susceptibility loci in approximately half of the current sample have been reported elsewhere (Ober et al. 1998, 1999). Here we report the results of a second-generation genomewide screen using 563 markers that include both microsatellites and single-nucleotide polymorphisms (SNPs) in candidate genes, for asthma- and atopy-susceptibility loci in the full sample of 693 Hutterites.

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Subjects and Methods

Sample Composition and Evaluation of Phenotypes

The study subjects are 693 Hutterites who live on nine communal farms (colonies) in South Dakota. Approximately half of these individuals (from four colonies) were the subjects of our previous genome scans (Ober et al. 1998, 1999). The nine colonies represent three of the four lines of the S-leut Hutterite colony lineages (Mange 1964; Ober et al. 1997). All Hutterites >5 years old who were in the colony on the days of our visits were included in these studies. The mean age of the participants is 28.7 years (SD 17.0 years; range 6–89 years), and the male:female ratio is 321:372. The mean inbreeding coefficient of the individuals in this sample is .034 (SD .015), slightly greater than that of first cousins once removed. However, because we do not know the relationships between all 64 ancestors and because some could have been related to one another, this may be an underestimate of the true inbreeding level in this population (discussed in Ober et al. 1997).

Subjects were evaluated using a modified version of the Collaborative Study on the Genetics of Asthma (CSGA) protocol (The Collaborative Study on the Genetics of Asthma 1997), as described elsewhere (Ober et al. 1998, 1999). In brief, a strict diagnosis of asthma was based on the presence of both self-reported symptoms (at least two of the following three: cough, wheeze, or shortness of breath) and bronchial hyperresponsiveness (BHR) ($\geq$20% decrease in baseline forced expiratory volume in 1 s [FEV_1] after inhalation of $\leq$25 mg methacholine/ml). A second, more relaxed, asthma diagnosis (referred to as "loose asthma") included individuals with either symptoms or BHR. Atopy was assessed by skin-prick test (SPT) using the following common allergens: house-dust mites (HDM) (*Der pteronyssinus* and *D. farinae*), cockroaches (*Blattella germanica* and *Periplaneta americana*), molds (*Alternaria alternata*, *Cladosporium herbarum*, and *Aspergillus fumigatus*), pollens (*Lolium perenne*, *Ambrosia artemisifolia*, *Artemisia vulgaris*, *Quercus alba*, and *Betula verrucosa*), and animal dander (*Felis domesticus* and *Canis familiaris*), as well as negative (saline) and positive (histamine) controls. An SPT was considered positive if the mean diameter of the wheal size was $\geq$3 mm larger than the mean diameter of the negative control (saline), as described elsewhere (Ober et al. 1999). Genetic studies of asthma-associated quantitative traits—including IgE, FEV_1, eosinophilia, and a quantitative measure of BHR—using a multipoint variance-component approach, have been presented elsewhere (Abney et al. 2000*b*).

Statistical Analysis

A complete genealogy of the 693 individuals was constructed from a >12,000-member Hutterite pedigree. This yielded a 1,623-person pedigree that included all known ancestors of the 693 individuals. For the genome screen, this large pedigree was divided into 20 subpedigrees for asthma (mean size 55 individuals, range 30–79) and 10 for atopy (mean size 118 people, range 53–209); all inbreeding loops were trimmed, and no meiosis was represented in more than one subpedigree. The large sizes of these pedigrees precluded the use of multipoint analyses. Instead, the evidence for linkage was examined using a maximized likelihood in which the penetrances, disease-susceptibility allele frequency, and recombination frequency are free parameters, although the penetrances and allele frequencies are constrained by the population prevalence of disease. The maximized likelihood is compared with one in which the penetrances and disease-susceptibility allele frequency are free but the recombination frequency is constrained to .5 (Curtis and Sham 1995). A likelihood ratio (LR) χ^2 with a 50:50 mix of a χ^2 distribution, with 1 df and point mass set at 0, can be used to assess the evidence for linkage. The advantage of this approach is that a mode of inheritance for asthma or SPT does not need to be specified, and, in this sense, the test is semiparametric. However, because the inbreeding information is not included, it is likely that considerable power is sacrificed.

As a second assessment of linkage among the Hutterites, we used the transmission/disequilibrium test (TDT) (Spielman et al. 1993; Spielman and Ewens 1996) in trios of affected individuals and their parents. Because the Hutterites are members of a single large pedigree and because all individuals are related to one another, the TDT is valid only for testing of linkage in the presence of disequilibrium, not for testing of disequilibrium in the presence of linkage (Zheng and Elston 1999). Because linkage disequilibrium with short tandem repeat polymorphisms can extend to 5 cM in the Hutterites (Hall et al. 1997), we used the TDT with framework markers in our genome scan, as discussed elsewhere (Ober et al. 1998, 1999).

The large size of the Hutterite subpedigrees and the corresponding computational requirements of linkage analysis made it impractical to perform simulations to assess genomewide significance. Instead, we present results on regions that show evidence suggestive of linkage, according to recommended criteria ($P \leq .001$) (Lander and Kruglyak 1995). However, we focus our discussion on regions that show evidence for linkage by both the LR test and the TDT in the Hutterites and on regions that show evidence for linkage among the Hutterites and

Table 1

Clinical Characteristics of a Hutterite Sample, by Asthma Affection Status

Subject Characteristic	Unaffected (n = 414)	Symptoms Only (n = 73)	BHR Only (n = 80)	Symptoms + BHR (Strict Asthma) (n = 71)	Total Sample (n = 638)
Mean age [SD] (years)	28.8 [15.5]	31.2 [15.5]	24.7 [19.3]	22.51 [15.9]	27.9 [16.1]
M:F	.78	.92	.90	1.63	.90
Mean log IgE [SD] (IU)	1.3 [.7]	1.5 [.8]	1.5 [.8]	1.8 [.8]	1.4 [.71]
+SPT (%):[a]					
⩾1 Allergen	41.8	52.0	50.0	52.1	45.1
⩾2 Allergens	22.5	26.0	27.5	32.4	24.6
HDM	21.3	21.9	32.5	29.6	23.7
Cockroach	19.8	23.3	28.8	19.7	21.3
Molds	9.9	11.0	11.3	16.9	11.0
Pollens/trees	20.0	28.8	18.8	36.6	22.7
Animal danders	6.5	8.2	11.3	12.7	8.0

NOTE.—Fifty-five individuals with genome screens were not included in this table, for the following reasons: 17 individuals (12 unaffected and 5 with strict asthma) did not have skin testing or IgE studies; 38 individuals could not be assigned an affection status, because they did not undergo methacholine-challenge testing for BHR, either because of pregnancy (n = 3) or illness (n = 1), because they were too young (n = 10) or too old (n = 14) to perform the maneuvers on the day of our visit, or because they were noncompliant (n = 10). None of the 38 untested individuals reported symptoms of asthma.

[a] Taxa used in tests are as follows: HDM—*D. pteronyssinus* and *D. farinae;* cockroach—*B. germanica* and *P. americana;* molds—*A. alternata*, *C. herbarum*, and *A. fumigatus;* pollens/trees—*L. perenne*, *A. artemisifolia*, *A. vulgaris*, *Q. alba*, and *B. verrucosa;* animal danders—*F. domesticus* and *C. familiaris*.

in other independent samples, because these are much more likely to be true linkages and to contain asthma- or atopy-susceptibility loci.

Analyses were performed for four diagnostic categories of asthma: strict asthma (BHR and symptoms) (n = 71), BHR (n = 151), symptoms (n = 144), and loose asthma (BHR or symptoms) (n = 224). Analyses were performed for five atopy categories: positive SPT to more than one allergen group (n = 311), positive SPT to HDM (n = 158), positive SPT to cockroach (n = 148), positive SPT to mold (n = 75), and positive SPT to pollens/trees (n = 156). Because so few Hutterites (n = 54) had positive SPTs to animal danders, we did not consider this as a separate category.

Genotyping

A genome screen using 386 microsatellite markers (screening set 9) was completed by the Mammalian Genotyping Service of the National Heart, Lung and Blood Institute, yielding a 9.1-cM map. Subjects were genotyped for 177 additional markers in selected regions of the genome, including polymorphisms in 17 candidate genes. These "candidates" were selected either because they were located in regions that showed evidence of linkage to asthma, atopy, or a related phenotype in our earlier or current study or because they were reported by others to be associated with asthma or atopy or both.

Genotypes at microsatellite loci were determined by a fluorescent-based system; all map distances are based on the Marshfield map. Genotyping protocols and map distances can be obtained from the Center for Medical Genetics, Marshfield Medical Research Foundation, Web site. The methods for genotyping polymorphisms in the *B2AR*, *IL4RA*, and HLA class II genes have been described elsewhere (Weitkamp and Ober 1999; Ober et al. 2000; Summerhill et al. 2000). SNPs in the *IL4*, *IL13*, and *CD14* genes were genotyped, either by using allele-specific oligonucleotide probes in dot-blotted DNA or by using denaturing high-performance liquid chromatography, according to standard protocols (e.g., those described in Ober et al. 2000).

Results

The clinical characteristics of our study sample are shown in table 1. The prevalence of strict asthma among the Hutterites is 11.6%; an additional 12.2% of individuals had asymptomatic BHR, and 11.1% reported asthma symptoms in the absence of BHR. The prevalence of atopy (defined as more than one positive SPT) ranges from 41.8% in unaffected individuals to 52.1% in asthmatics. It is notable that the prevalence of atopy is low in Hutterites with asthma, compared with that in outbred white asthmatic populations, in which the prevalence of atopy is ~80% (Holt et al. 1999; The Collaborative Study on the Genetics of Asthma, unpublished data).

The linkage results at all loci are available on the Web site Complex Trait Mapping in the Hutterites. Twenty-three loci in 18 chromosomal regions showed evidence

for linkage at $P \leq .001$, by either the LR χ^2 test or the TDT (table 2), which met the criteria for suggestive linkage that have been proposed by Lander and Kruglyak (1995). These regions overlap with some of the regions identified in our earlier genome screens, which used less-stringent criteria ($P < .01$) (Ober et al. 1998, 1999). However, among the six regions shown in table 2 that demonstrate some evidence for linkage by both the LR χ^2 test and the TDT, only one region, on 19q (D19S900 with BHR; LR χ^2 $P = .0010$, TDT $P = .0072$), also showed evidence for linkage in our earlier studies. The remaining five regions were not detected previously: 5p (D5S1470 with BHR [$P = .0010$ by LR χ^2, $P = .0061$ by TDT]); 5q (D5S1462 with loose asthma [$P = .0007$ by LR χ^2, $P = .028$ by TDT]); 8p (D8S1136 with strict asthma [$P = .0009$ by LR χ^2, $P = .039$ by TDT]), 14q (gata193a07 with loose asthma [$P = .019$ by LR χ^2, $P = .0001$ by TDT]), and 16q (D16S539 with positive SPT for molds [$P = .0008$ by LR χ^2, $P = .029$ by TDT]).

Four additional regions that showed evidence for linkage in our previous studies show strong evidence

Table 2

Results of Genomewide Screen

Chromosome and Locus	Distance from pter (cM)	*P* for[a]		Allele (bp)	TR:NT	Phenotype
		LR χ^2	TDT			
1:						
D1S468	4	.0002	NS	...	...	Strict asthma
D1S1597	30	.0008	NS	...	...	Loose asthma
D1S3669	37	.0005	NS	...	...	Strict asthma
D1S239[b]	139	NS	.0005	242	52:22	+SPT cockroach
2:						
D2S2944	210	NS	4×10^{-5}	115	57:21	+SPT cockroach
		.0009	NS	...	...	+SPT HDM
3:						
D3S3564[b]	68	.0004	NS	...	...	Loose asthma
D3S2459	119	.0007	NS	...	...	+SPT mold
5:						
D5S1470	**45**	**.0010**	**.0061**	**177**	**25:9**	**BHR**
D5S1462	**105**	**.0007**	**.028**	**193**	**24:11**	**Loose asthma**
D5S1453[b]	115	.0010	NS	...	...	Symptoms
D5S2014[b]	153	.0009	NS	...	...	Symptoms
8:						
D8S1477	60	.0004	NS	...	...	Strict asthma
D8S1110	67	.0006	NS	...	...	Strict asthma
D8S1136	**82**	**.0009**	**.039**	**257**	**14:5**	**Strict asthma**
D8S2324	94	.0007	NS	...	...	+SPT
9:						
D9S938	111	NS	.0005	409	44:17	Symptoms
11:						
D11S899[b]	23	.0003	NS	...	...	+SPT mold
13:						
D13S787[b]	9	.0006	NS	...	...	Symptoms
14:						
gata193a07	**96**	**.019**	**.0001**	**343**	**51:19**	**Loose asthma**
16:						
D16S539	**125**	**.0008**	**.029**	**152**	**78:53**	**+SPT mold**
18:						
ADYCYAP1	0.6	.0002	NS	...	...	Symptoms
19:						
D19S900[b]	67	**.0010**	**.0072**	**165**	**41:20**	**BHR**
D19S540[b]	70	.0004	NS	...	...	BHR
20:						
D20S470	39	NS	.001	298	16:2	+SPT HDM

Note.— All loci with $P \leq .001$ by either the LR χ^2 test or the TDT are shown for phenotypes with the most significant *P* value at that locus (for definitions of phenotypes see Subjects and Methods). Six loci showing some evidence for linkage by both tests are shown in boldface.

[a] NS = not significant ($P \geq .05$).

[b] Region (defined by 20 cM) identified in earlier studies (Ober et al. 1998, 1999).

Table 3

Results of the TDT for Polymorphisms in Candidate Genes

Gene[a]	Region	Polymorphism(s)	Associated Phenotype	P[b]	Associated Allele or Haplotype	TR:NT
CTLA4	2q31-32	AT repeat in 3′UTR	None	NS	…	…
IL4	5q31	nt −590(C/T)	None	NS	…	…
		nt 8374(A/G)	None	NS	…	…
		Haplotype	None	NS	…	…
IL13	5q31	Arg110Glu	None	NS	…	…
IL9	5q32	GT repeat in intron 4	None	NS	…	…
CD14	5q32	nt −159 (C/T)	**≥1 +SPT**	**.00091**	**−159T**	**110:66**
ADRB2R[c]	5q33	Arg16Gly	None	NS	…	…
		Gln27Glu	None	NS	…	…
		Haplotype	None	NS	…	…
TNFa	6p21	GT repeat 250 kb upstream of *LTA*	None	NS	…	…
HLA-DRB1[d]	6p21	DRB1	**+SPT cockroach**	**.00059**	***0101**	**66:32**
HLA-DQA1[d]	6p21	DQA1	**+SPT cockroach**	**.00017**	***0101**	**70:32**
HLA-DQB1[d]	6p21	DQB1	**+SPT cockroach**	**.00012**	***0501**	**71:32**
IFNA	9p22	GT repeat in proximal half of IFN cluster	**Symptoms**	**.0019**	**104 bp**	**48:22**
FCER1B	11q13	CA repeat in intron 5	None	NS	…	…
IFNG	12q24	CA repeat in intron 1	≥1 +SPT	.013	185 bp	39:20
IGF1	12q24	GT repeat in promoter region	BHR	.033	196 bp	16:6
NOS1	12q24	AAT repeat in intron	None	NS	…	…
IL4RA[e]	16p12	Cys406Arg	**≥1 +SPT**	**.0051**	**Cys406**	**60:33**
		Haplotype	**Strict asthma**	**.00061**	**Glu375-Ser478**	**27:7**
IFNAR	21q22	GT repeat in 5′UTR	None	NS	…	…

NOTE.—Only the most significant result at each locus is shown; regions demonstrating strong evidence for linkage are shown in boldface.

[a] *CTLA4* = cytotoxic T lymphocyte antigen-4 (MIM 123890); *IL4* = interleukin-4 (MIM 147780); *IL13* = interleukin-13 (MIM 147683); *IL9* = interleukin-9 (MIM 146931); *CD14* = monocyte differentiation antigen CD14 (MIM 158120); *ADRB2R* = β_2-adrenergic receptor (MIM 109690); *TNFa* = tumor necrosis factor–a; *IFNA* = interferon-α (MIM 147660); *LTA* = microsatellite marker upstream to lymphotoxin A gene (MIM 153440); *FCER1B* = Fcϵ receptor–β_1 (MIM 147148); *IFNG* = interferon-γ (MIM 147570); *IGF1* = insulin-like growth factor–1 (MIM 147440); *NOS1* = nitrous oxide synthase–1 (MIM 163731); *IL4RA* = IL-4 receptor α-chain (MIM 147781); *IFNAR* = interferon-α receptor (MIM 107451).

[b] NS = Not significant ($P > .05$).

[c] For additional details, see Summerhill et al. (2000).

[d] For additional details, see Donfack et al. (2000).

[e] For additional details, see Ober et al. (2000).

for linkage in the present study ($P \leq .001$), by either the LR χ^2 test or the TDT (table 2). These include regions on 1p (D1S239 with positive SPT to cockroach [$P = .0005$ by TDT]), 3p (D3S3564 with loose asthma, [$P = .0007$ by χ^2]), 5q (D5S2014 with symptoms [$P = .0009$ by χ^2]), and 13q (D13S787 with symptoms [$P = .0006$ by χ^2]). Finally, seven regions that showed some evidence for linkage in our earlier studies continue to show modest evidence of linkage ($P < .01$) in the present study (data not shown). These include regions on 1p (positive SPT to mold), 2q (loose asthma), 4q (positive SPT to pollens/trees), 5q (positive SPT to cockroach), 9p (BHR and positive SPT to mold), 12q (loose asthma and positive SPT to cockroach), and 21q (positive SPT to HDM). Thus, 12 regions that were identified in our earlier studies continue to show some evidence for linkage in this expanded study. In addition, results in the present study identified 11 new regions that show evidence for linkage when the more stringent criterion of $P < .001$ is used.

The results of the TDT for 21 polymorphisms in 17 candidate genes are shown in table 3. These candidates were selected, either because they were in regions that showed linkage in our earlier or present studies (2q, 5q, 6, 9p, 12q, 16p, and 21q), because they were associated with asthma, atopy, or a related phenotype in other studies (*FCER1B*), or because they were both (*IL4, IL13, CD14, ADRB2R, TNF, HLA, NOS1,* and *IL4RA*) (reviewed in Ober and Moffatt 2000). Detailed studies of the *B2AR*, *IL4RA*, and HLA class II genes in the Hutterites have been published elsewhere (Donfack et al. 2000; Ober et al. 2000; Summerhill et al. 2000). Alleles at two additional loci were significantly overtransmitted to affected individuals, suggesting linkage and possible association. The CD14-159T allele was overtransmitted to atopic individuals (110 transmis-

Table 4

Regions Showing Evidence for Linkage to Asthma, Atopy, or Related Phenotypes among the Hutterites (Table 2) and in Other Genome Screens

Chromosome	Distance from pter (cM)	Present Study	Busselton[a]	United States[b]	Germany[c]	France[d]
1	4–14	Asthma	…	…	Specific IgE (P = .0013)	…
	30–37	Asthma	…	…	Eosinophils (P = .009)	…
	139–150	+SPT	…	…	IgE, specific IgE (P = .0098, .0045)	…
2	186–215	+SPT	…	…	IgE (P = .0016)	…
5	33–52	BHR	…	…	Specific IgE (P = .0027)	…
9	95–112	Symptoms	…	…	Asthma (P = .0073)	…
11	21–43	+SPT	…	Asthma (LOD = 1.2)[e]	Specific IgE (P = .0036)	IgE (P=.002)
16	105–125	+SPT	IgE (P < .01)	…	…	…
19	67–70	BHR	…	Asthma (LOD = 2.0)[e]	…	BHR (P = .01)
20	32–39	+SPT	Wheeze (P < .01)	…	…	…

NOTE.—Only values P < .01 are shown. A review of all of the regions of overlap between the five genomewide screens is presented elsewhere (Ober and Moffatt 2000).

[a] Source: Daniels et al. (1996).

[b] Sources: The Collaborative Study on the Genetics of Asthma (1997); Mathias et al. (2000).

[c] Sources: Wjst (1999); Wjst et al. (1999).

[d] Source: Dizier et al. (2000).

[e] In white families.

sions vs. 66 nontransmissions; P = .0009), and the IFNA 104-bp allele was overtransmitted to symptomatic individuals (48 transmissions vs. 22 nontransmissions; P = .0019). The modest evidence for overtransmission of alleles at the *IFNG* and *IGF1* loci on 12q probably reflects linkage disequilibrium with susceptibility loci in these regions, because other nearby markers show as much (or more) evidence of nonrandom transmission (see the Web site Complex Trait Mapping in the Hutterites). Polymorphisms in the remaining genes did not show evidence of association in the present study.

Discussion

The Hutterites have been nearly completely reproductively isolated for >200 years. Few individuals have joined their community since its founders immigrated to the United States during the 1870s, and all 64 of the ancestors of our pedigree with asthma were born in Russia between the early 18th and early 19th centuries. Phenotypically, the asthma that is present in the Hutterites is milder than that observed in outbred populations in the United States: none of the individuals in our study used inhaled steroids for treatment of their symptoms, and hospitalizations for exacerbation of symptoms are rare. Furthermore, only ~50% of Hutterites with either asthma or BHR were atopic, which is a considerably smaller proportion than that observed in other white asthmatic populations (Holt et al. 1999; The Collaborative Study on the Genetics of Asthma, unpublished data). Nonetheless, six of the regions with evidence for linkage to asthma phenotypes and five with evidence for linkage to atopy in the Hutterites (table 1) have also shown evidence for linkage to a related phenotype in other genomewide screens (table 4), indicating that many of the same loci influence susceptibility in the Hutterites and in outbred populations. This would further suggest that the susceptibility alleles in these regions are common variants, as discussed elsewhere (Ober et al. 1998), and are therefore similar to known risk alleles for other common diseases (reviewed by Wright et al. 1999).

Genomewide screens for asthma- and atopy-susceptibility alleles have been reported for four population samples in addition to the Hutterites: the Busselton (Australian) population (Daniels et al. 1996), the U.S. CSGA families (The Collaborative Study on the Genetics of Asthma 1997; Mathias et al. 2000), a German population (Wjst 1999; Wjst et al. 1999), and a French population (Dizier et al. 2000). Ten of the regions showing evidence for linkage among the Hutterites also show some evidence of linkage (P < .01) to a related phenotype in at least one of these outbred samples (table 4). Remarkably, a 20-cM region on 11p has been linked to either asthma or atopy in four of the five samples, and a region on 19q has been linked in three of five population samples. It is not surprising that the Hutterites and a sample from Germany share the most linked regions, because the Hutterite population that emigrated from Russia was largely of German ancestry. Taken together, these data implicate loci in 10 genomic regions as having susceptibility alleles that are present in diverse population samples and as having, perhaps,

relatively large effects on asthma or atopy phenotypes. The fact that there are not more regions of overlap between the Hutterites and the outbred samples may reflect (1) differences in the definition of phenotypes, as in the Busselton study; (2) fewer phenotypes analyzed, as in the CSGA; (3) true genetic heterogeneity between the Hutterites and the non-Germanic outbred samples; or (4) the relatively imprecise localization of linked regions in genomewide screens with widely spaced (10-cM) markers. As the linked regions become better defined in each of these populations, particularly when the susceptibility loci within these regions are identified, it is likely that there will be more overlap between these samples than is apparent from the genomewide screens.

The specific susceptibility loci in these regions have not yet been identified. In a study reported elsewhere, we found significant associations between asthma and variation in the *IL4RA* locus on 16p12 and between positive SPT to cockroach and variation at the *HLA-DRB1* locus on 6p21 both among the Hutterites and among outbred CSGA families (Donfack et al. 2000; Ober et al. 2000) after genomewide screens that showed evidence for linkage in these regions (Ober et al. 1998, 1999). Surprisingly, markers on 16p12 and 6p21 did not show evidence for linkage in this second-generation genome screen. Because tests of association will more likely detect loci with small effects than will tests of linkage (Risch and Merikangas 1996), we interpret these data to indicate that *IL4RA* and *HLA-DRB1* are minor susceptibility loci for asthma and atopy, respectively, among the Hutterites.

In the present study, two other candidate genes showed evidence of significant nonrandom transmission to affected individuals. The *CD14* locus encodes the high-affinity receptor for lipospolysaccharide and is in the cytokine gene cluster on 5q, a region that shows evidence for linkage among the Hutterites and in many outbred populations (Marsh et al. 1994; Meyers et al. 1994; Daniels et al. 1996; Doull et al. 1996; The Collaborative Study on the Genetics of Asthma 1997; Noguchi et al. 1997; Hizawa et al. 1998; Martinez et al. 1998; Palmer et al. 1998). Previous studies in outbred white populations have reported associations between the CD14-159C allele and elevated IgE levels (a marker for atopy) (Baldini et al. 1999; Gao et al. 1999). The *CD14* locus is ~4 cm from D5S642, a marker showing evidence of linkage to atopy in the Hutterites (TDT; $P < .01$) (Ober et al. 1999). Interestingly, although the *CD14* –159T allele was overtransmitted to Hutterites with atopy (table 3), overtransmission occurred only when the allele was on a haplotype with the D5S642 185-bp allele (transmitted:nontransmitted [TR:NT] 34:14) and not when it is on other haplotypes (TR:NT 36:45). This finding suggests that in the Hutterites the *CD14* −159T allele is in linkage disequilibrium with the true susceptibility variant, which is likely to be within or closely linked to the *CD14* locus on 5q.

The type I IFN gene cluster encompasses a region of ~400 kb on chromosome 9p22 and contains 13 *IFN-α* genes, a single locus encoding IFN-*β*, and 11 IFN pseudogenes (Diaz 1995). The GT-repeat polymorphism reported here is in the proximal half of the cluster, close to six *IFN-α* genes (Golovleva et al. 1996), and has been associated with risk for multiple sclerosis (Miterski et al. 1999). In our study, a 148-bp allele was significantly overtransmitted to affected individuals (table 3), and the 150- and 146-bp alleles were significantly undertransmitted to affected individuals. This yielded a highly significant global TDT ($P = 2.5 \times 10^{-5}$) and suggested that alleles at a locus in this region confer susceptibility to—and, perhaps, protection against—asthma. *IFN-α* genes have not previously been reported to be associated with asthma, and there has not been compelling evidence for linkage in this region, indicating that this may also be a minor asthma-susceptibility locus.

In this second-generation genome screen for asthma- and atopy-susceptibility alleles in the Hutterites, the evidence that we detected for linkage was, in general, 10-fold more significant than the evidence in our previous reports. The larger sample size ($n = 693$ vs. $n = 361$) probably contributed to this increase in power. Furthermore, although many of the regions that showed the most evidence for linkage in our previous studies continue to show some evidence for linkage in the present study, nearly all of the regions showing the most evidence for linkage here were not detected in our earlier studies. In particular, five of the six regions showing evidence for linkage, by both the LR χ^2 test and the TDT, in the present study did not show evidence for linkage in our previous studies. This may not be surprising, given the potentially large number of loci that influence susceptibility, even in the Hutterites, and given the fact that most susceptibility loci probably have relatively modest effects on risk (e.g., Suarez et al. 1994). However, these results argue against the use of a two-stage genome screen. Linkages may be missed when only a subset of the sample is genotyped using widely spaced markers (~20 cM) and then the entire sample is genotyped using additional markers only in regions identified as "interesting" in the first stage (Elston et al. 1996). Although such an approach may be useful under some simple or oligogenic genetic models, it may not be appropriate for phenotypes, such as asthma, that are influenced by variation at many genetic loci. If we had used that strategy in the present study, all but one of the most significant linkages would have been missed.

In summary, these data, combined with other published genome screens, suggest that regions on 11p and 19q contain loci that may have relatively large effects

on asthma susceptibility in diverse population samples. In the context of this genome screen, we can further conclude that *IL4RA* on 16p and *HLA-DRB1* on 6p are minor asthma- and atopy-susceptibility loci, as may be true for an *IFNA*-linked locus on chromosome 9p. Variation either in or closely linked to the *CD14* gene on 5q may account for some of the linkages to atopy that have been reported for markers in this region in the Hutterites and other populations, but this remains to be proved.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/

Complex Trait Mapping in the Hutterites, http://www.genes.uchicago.edu/hutterite/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *CTLA4* [MIM 123890], *IL4* [MIM 147780], *IL13* [MIM 147683], *IL9* [MIM 146931], *CD14* [MIM 158120], *ADRB2R* [MIM 109690], *LTA* [MIM 153440], *IFNA* [MIM 147660], *FCER1B* [MIM 147148], *IFNG* [MIM147570], *IGF1* [MIM 147440], *NOS1* [MIM 163731], *IL4RA* [MIM 147781], *IFNAR* [MIM 107451])

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