

Fine mapping and positional candidate studies on chromosome 5p13 identify multiple asthma susceptibility loci

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Background: Genome-wide linkage scans to identify asthma susceptibility loci have revealed many linked regions, including a broad region on chromosome 5p.

Objective: To identify a 5p-linked asthma or bronchial hyperresponsiveness (BHR) locus.

Methods: We performed fine mapping and positional candidate studies of this region in the Hutterites and an outbred case-control sample from Germany by genotyping 89 single nucleotide polymorphisms (SNPs) in 22 genes. SNP and haplotype analyses were performed.

Results: Three genes in a distal region (zinc finger RNA binding protein [ZFR], natriuretic peptide receptor C, and a disintegrin and metalloproteinase domain with thrombospondin type 1 motif [ADAMTS12]) were associated with BHR, whereas 4 genes in a proximal region (prolactin receptor, IL-7 receptor [IL7R], leukemia inhibitory factor receptor [LIFR], and prostaglandin E4 receptor [PTGER4]) were associated with asthma symptoms in the Hutterites. Furthermore, nearly the entire original linkage signal in the Hutterites was generated by individuals who had the risk-associated alleles in ZFR3, natriuretic peptide receptor C, ADAMTS12, LIFR, and PTGER4. Variation in ADAMTS12, IL7R, and PTGER4 were also associated with asthma in the outbred Germans, and the frequencies of long-range haplotypes composed of SNPs at ZFR, ADAMTS12, IL7R, LIFR, and PTGER4 were significantly different between both the German and Hutterite cases and controls. There is little linkage disequilibrium between alleles in these 2 regions in either population.

Conclusion: These results suggest that a broad region on 5p, separated by >9 Mb, harbors at least 2 and possibly 5 asthma or BHR susceptibility loci. These findings are consistent with

the hypothesis that regions providing evidence for linkage in multiple populations may, in fact, house more than 1 susceptibility locus, as appears to be the case for the linked region on 5p.

Clinical implications: Identifying asthma or BHR genes could lead to novel therapeutic approaches. (*J Allergy Clin Immunol* 2006;118:396-402.)

Key words: Asthma, linkage, founder population

Asthma is among the most common chronic diseases in developed countries, affecting nearly 155 million people worldwide.¹⁻³ Family and twin studies have suggested a significant genetic component,^{4,5} although many environmental factors have also been implicated in disease risk.⁶⁻¹⁰ This complex etiology, combined with extensive heterogeneity, has made genetic studies challenging. Not surprisingly, linkages to many regions have been reported, implicating a minimum of 25 genes in asthma pathogenesis (see review¹¹). Recently, positional cloning studies have identified genes in 6 of these regions that confer susceptibility to asthma, bronchial hyperresponsiveness (BHR), or atopy.¹²⁻¹⁷

To identify asthma susceptibility loci, we conducted a genome-wide screen for asthma and related phenotypes in the Hutterites, a young founder population.¹⁸ The microsatellite marker D5S1470 on chromosome 5p showed evidence for linkage to BHR by 2 measures, the likelihood ratio (LR) χ^2 test ($\chi^2 = 9.52$; $P = .001$) and the transmission disequilibrium test (TDT; 177-bp allele, 25 transmissions: 9 nontransmissions; $\chi^2 = 7.53$; $P = .0061$).¹⁸ The evidence for linkage by the TDT fell off with flanking markers beyond 1 cM, suggesting that a disease gene is located within a ~2 cM region that includes D5S1470. The evidence for linkage by the LR test was more extensive, as expected, spanning nearly 20 cM. Markers in this broader region also showed evidence for linkage to asthma and asthma symptoms ($P < .05$) in the Hutterites, to asthma in the Collaborative Study on the Genetics of Asthma (CSGA) African American families,¹⁹ to serum IgE levels in German families,²⁰ to BHR in French families,²¹ and to sensitization to house dust mites in European families.²² These combined data suggested that 1 or more loci on chromosome 5p influence susceptibility to asthma-related or atopy-related phenotypes. We present here results of fine mapping and positional candidate studies of this

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Abbreviations used

ADAM33:	A disintegrin and metalloproteinase domain 33
ADAMTS12:	A disintegrin and metalloproteinase domain with thrombospondin type 1 motif 12
BHR:	Bronchial hyperresponsiveness
EP:	Prostaglandin E
HWE:	Hardy-Weinberg equilibrium
IL7R:	IL-7 receptor
LD:	Linkage disequilibrium
LIFR:	Leukemia inhibitory factor receptor
LR:	Likelihood ratio
NPR3:	Natriuretic peptide receptor C
PRLR:	Prolactin receptor
PTGER4:	Prostaglandin E4 receptor
SNP:	Single nucleotide polymorphism
TDT:	Transmission disequilibrium test
ZFR:	Zinc finger RNA binding protein

region in the Hutterites and in an outbred case-control sample from Germany. Our results suggest that chromosome 5p13 harbors at least 2 asthma susceptibility loci.

METHODS

Sample composition and evaluation of phenotypes

Fine mapping studies were conducted in 693 Hutterites who live on 9 communal farms in South Dakota (for details, see reference¹⁸). These individuals are related to each other in a single 13-generation, 1623-person pedigree. All members of this pedigree can be traced back to 62 founders who lived during the early 1700s to early 1800s,^{23,24} although the Hutterites originated 2 centuries earlier in the Tyrolean Alps and still retain a German dialect as their first language.²⁵

Hutterites were evaluated by using a modified version of the Collaborative Study on the Genetics of Asthma protocol,¹⁹ as previously described.¹⁸ In brief, BHR was defined as a $\geq 20\%$ decrease in baseline FEV₁ after inhalation of ≤ 25 mg/mL methacholine. The diagnosis of asthma was based on the presence of self-reported symptoms (at least 2 of the following 3: cough, wheeze, and shortness of breath), a doctor's diagnosis, and BHR. One hundred fifty-six Hutterites had BHR, and 149 had asthma symptoms; 76 had both and were diagnosed with asthma. Because it is likely that asthma was underdiagnosed in the Hutterites (see discussion²⁶), all association studies were performed considering each of 3 phenotypes (asthma symptoms, asthma, and BHR).

For follow-up studies and to facilitate localization, a case-control sample was ascertained in southwest Germany. Between July 2000 and October 2001, 321 children with suspected asthma (age 5-18 years) were recruited from clinics at the Children's University Hospital in Freiburg, Germany. Asthma was diagnosed according to the American Thoracic Society criteria.²⁷ BHR was defined as a fall in baseline FEV₁ by 15% after either inhalation of ≤ 8 mg/dL histamine or ≤ 6 minutes of exercise provocation. The diagnosis of asthma was based on the presence of both self-reported symptoms (at least 1 of the following 3: cough, wheeze, and shortness of breath), use of asthma medication, and BHR, similar to the criteria used in the Hutterites. Two hundred thirty-one of the 321 children who were

screened were subsequently diagnosed with asthma. The mean age of the asthma cases was 10.8 years (range, 5-18 years); the male to female ratio was 2:1.

Two hundred seventy unrelated individuals were recruited from the same region in southwestern part of Germany as control subjects. No medical history was taken, and no medical testing was performed on these individuals. We recognize that using unphenotyped population samples as controls, rather than controls without asthma, leads to a reduction in power. However, this reduction is very small (on average $<4\%$; data not shown), and it allows for an unbiased estimation of allele frequencies, genotype relative risks, and odds ratios in the population.

These studies were approved by the University of Chicago and University of Freiburg Institutional Review Boards.

Genotyping

After a genome screen,¹⁸ a list of all genes of the region flanking D5S1470 was compiled by using public databases (<http://genome.ucsc.edu>; see this article's Table E1 in the Online Repository at www.jacionline.org). The original evidence for linkage by the TDT included markers that spanned a ~ 2 -Mb region. This region is relatively gene-poor, with only 9 known or putative genes (average, 1 gene per 232 kb). Our goal was to genotype common single nucleotide polymorphisms (SNPs; minor allele frequency > 0.10) in each gene in this 2-Mb region that was documented as of the May 2004 (hg17) release. Additional typing was performed to achieve an average spacing of ≤ 20 kb between SNPs and to include all nonsynonymous SNPs in genes that showed any evidence of association with the typed SNPs or haplotypes. In addition, because our linkage signal from the LR test and linkage signals in other populations spanned a broader region, we also examined SNPs in 5 functional candidates, 4 (IL-7 receptor [*IL7R*], leukemia inhibitory factor receptor [*LIFR*], prolactin receptor [*PRLR*], prostaglandin E4 receptor [*PTGER4*]) located as much as ~ 14 cM (~ 8 Mb) centromeric to D5S1470, and 1 (*CDH6*) located ~ 4 cM (~ 1.3 Mb) telomeric, and some flanking genes in each region to fill in gaps (see this article's Table E1 in the Online Repository at www.jacionline.org). We used this more inclusive strategy because weak linkage signals can be generated for distances that are tens of cM from the true susceptibility locus for phenotypes like asthma that are characterized by genetic heterogeneity, low penetrance, and high phenocopy rates.²⁸

Initially, SNPs were identified by denaturing HPLC²⁹ in DNA from 15 Hutterites carrying all combination of genotypes at D5S1470 and with different affection statuses. Eventually, however, all SNPs were selected from public databases. The presence and approximate frequency of SNPs identified by 1 of these 2 approaches were determined by sequencing pooled DNA from 15 affected (BHR) and 15 unaffected (no BHR, no symptoms) Hutterites carrying all combinations of genotypes at the D5S1470 locus. Confirmed SNPs with estimated minor allele frequencies > 0.10 were then genotyped in the 693 Hutterites using single base extension with fluorescence polarization,³⁰ restriction enzyme digestion, or DNAPrint Genomics (Sarasota, Fla), using a Beckman Coulter (Fullerton, Calif) GenomeLab SNPstream Genotyping System (<http://www.dnaprint.com/genotyping.html>).

Genotyping in the Freiburg sample was performed by restriction digestion in Freiburg, or by DNAPrint Genomics concurrently with genotyping in the Hutterites. Some SNPs typed in the Hutterites were not typed in the Germans because we excluded them as associated SNPs (or haplotypes) before the initiation of studies in the Germans.

Statistical analyses in the Hutterites

Genotyping errors in the Hutterites were detected by using the software PEDCHECK³¹ after subdividing the large multi-generational pedigree into smaller subpedigrees. Deviations from

Hardy-Weinberg equilibrium (HWE) were determined by using an approach allowing appropriately for related individuals.³²

To test for association, we used case-control tests that are designed specifically for large, multigenerational pedigrees and that are more powerful than the TDT to detect associations in the Hutterites.³³ In addition, we split the multigenerational pedigree into nuclear families and performed haplotype analysis by using the program FAMHAP, obtaining a global *P* value by permutation.^{34,35} Here, 1000 or 10,000 replicates were simulated for each *P* value, respectively. Linkage disequilibrium (LD) plots were generated by using publicly available software (<http://innateimmunity.net/IIPGA2/index.html>).

Because our ultimate goal was to identify the variation that generated the linkage signal on 5p, we evaluated the effect of genotypes at associated loci to determine whether alleles in specific genes partitioned the evidence for linkage to BHR at the marker D5S1470. For these studies, we implemented a method similar to that described by Sun et al³⁶ and to that used to identify calpain10 (*CAPN10*) as the noninsulin-dependent diabetes mellitus 1 (*NIDDM1*) gene.³⁷ The rationale for these approaches is as follows. Because we originally observed evidence for linkage with D5S1470 by the LR test, the disease variant should be segregating in those families that show evidence for linkage to D5S1470. When considering only families of affected individuals with 1 or 2 copies of associated alleles, the evidence for linkage should increase if the SNP accounts for the original evidence for linkage.³⁶ Likewise, the evidence for linkage should decrease when considering only families of affected individuals with no copies of the associated alleles.

For this analysis, the Hutterite pedigree was divided into 20 subpedigrees (mean, 55 individuals; range, 30–79) that included 154 BHR individuals, with all inbreeding loops removed and no meiosis represented in more than 1 subpedigree, as in our previous studies.¹⁸ The LR χ^2 in this sample was 8.147 (*P* = .004), which differed slightly from that previously reported¹⁸ because of modifications of the test used in this study and that in Ober et al.¹⁸ The LR χ^2 at D5S1470 was then recalculated after changing the affection status from affected (BHR) to unknown for individuals carrying the allele of interest and in the complementary group (ie, changing the BHR status to unknown for individuals without the allele of interest). This was repeated for each marker of interest. LR χ^2 tests were calculated by using MODLINK (<http://hardy.wustl.edu/download/modlink/modlink.htm>) to compare the maximized likelihood when the penetrances, disease-susceptibility allele frequency, and recombination frequency are free parameters to the maximized likelihood when recombination frequency is fixed at 0.5.³⁸ The penetrances and disease allele frequency were constrained by population prevalence of BHR. Because MODLINK searches for local, rather than global, maxima, we searched the likelihood space from 20 different starting positions for the parameters. If a partitioned set of individuals did not yield a likelihood maximum at $\theta \leq 0.3$, we fixed θ to 0.001, 0.01, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 and repeated the maximization analysis. If no reasonable maxima were again found (suggesting no evidence for linkage), we set the resultant χ^2 value at 0.

Because the studies in the Hutterites focus on both linkage and association mapping, insuring that results for nearby markers are correlated, it is inappropriately conservative to use Bonferroni corrections of *P* values for the number of comparisons. Thus, we rely instead on replication studies in the German sample both to confirm results detected in studies on the Hutterites and to improve the resolution of mapping.

Statistical analyses in the Freiburg sample

The program FINETTI (<http://ihg.gsf.de/linkage/download/finetti.zip>) was used to test for departures from HWE and for associations with asthma by the Pearson goodness-of-fit test. For the case-control

data, we performed haplotype analysis using the program FAMHAP,^{34,35} simulating 1000 or 10,000 replicates for each *P* value. LD plots were generated by using public available software (<http://innateimmunity.net/IIPGA2/index.html>).

RESULTS

Association studies in the Hutterites

A total of 82 SNPs in 22 genes were genotyped in the Hutterites, including common SNPs in each of the 11 genes in the 2-Mb region flanking D5S1470 (see this article's Table E1 in the Online Repository at www.jacionline.org). None of the SNPs showed departures from HWE (*P* > .01). The results of association studies with each SNP are shown in Fig 1. SNPs in 3 genes close to D5S1470 (zinc finger RNA binding protein [*ZFR*], natriuretic peptide receptor C [*NPR3*], a disintegrin and metalloproteinase domain with thrombospondin type 1 motif [*ADAMTS12*]) were associated with asthma or BHR (Fig 1, A). SNPs in 4 genes in the proximal region (*PRLR*, *IL7R*, *LIFR*, and *PTGER4*) were associated with asthma symptoms (Fig 1, B). The associated variants included nonsynonymous SNPs in *ZFR* (rs4867440, rs1051489), *IL7R* (rs1494558), and *LIFR* (rs3110234; red lines, Fig 1). Despite the evidence for association with SNPs in both the proximal (*PRLR*, *IL7R*, *LIFR*, and *PTGER*) and distal (*ZFR*, *NPR3*, and *ADAMTS12*) regions, there was little evidence for LD between alleles in these regions in the Hutterites (see this article's Fig E1 in the Online Repository at www.jacionline.org).

We next examined haplotypes comprised of SNPs within each of the 7 genes identified in the single SNP analyses (Fig 1). Significant differences in haplotype frequencies were observed for 5 of the 7 genes, including *ZFR* (*P* = .03), *NPR3* (*P* = .01), *ADAMTS12* (*P* = .002), *IL7R* (*P* = .04), and *LIFR* (*P* = .02). Moreover, long range multilocus haplotypes composed of SNPs in these 5 genes also revealed significant differences for an extended haplotype consisting of alleles at *ZFR*-9, *ZFR*-13, *IL7R*-9, and *LIFR*-11 (*P* = .0004).

Explaining the evidence for linkage in the Hutterites

To determine whether the associated variants could explain the original evidence for linkage at D5S1470 to BHR in the Hutterites, we calculated the LR χ^2 at D5S1470 after changing the BHR affection status to unknown for individuals with associated alleles and in the complementary group. For this analysis, we selected the most associated allele in the gene with a frequency between 0.10 and 0.90. Because the alleles at *ZFR* and *NPR* were in strong LD with the D5S1470-177 bp allele (mean *D'* = 0.84 and 0.65, respectively), we used the 177-bp allele in this analysis as a surrogate for the extended haplotype. We also included the *ADAMTS12*-3 (T), *PRLR*-1 (T), *LIFR*-4 (T), and *PTGER4*-1 (T) alleles in these studies. None of the associated *IL7R* SNPs met the allele frequency criterion, so they were not included in this analysis.

The results of the partitioning analysis are shown in Table I. Presence of the D5S1470 (177-bp) allele by itself was not sufficient to generate the evidence for linkage observed in the baseline analysis, but treating individuals with D5S1470 (177-bp) as unknown reduces the LR χ^2 from 8.147 to 4.933. The same pattern is observed with *ADAMTS12*-3 (T), likely reflecting the LD between these 2 alleles ($r^2 = 0.325$; $D' = 0.758$). The reduction of the LR χ^2 is expected in part because of the reduction in sample size in the partitioned analyses. However, we note that the sample sizes for the analyses excluding these individuals are still among the largest presented in Table I. Sizable LR χ^2 are observed for affected individuals with *LIFR*-4 (T) and *PTGER4*-1 (T) alleles (5.240 and 4.469, respectively), and changing their affection status to unknown reduces the LR χ^2 . This is particularly striking for *PTGER4*-1, where treating individuals with the T allele as unknown reduces the LR χ^2 from 8.147 to 1.420. Curiously, the opposite pattern is observed with *PRLR*-1 (T), for which removing affected individuals with this allele moderately increases the LR χ^2 , and it is severely reduced (to 0) when only individuals with that allele are considered affected. These results suggest that the linkage signal at D5S1470 is largely accounted for by families segregating alleles at D5S1470 (177-bp), *ADAMTS12*-3 (T), *LIFR*-4 (T), and *PTGER4*-1 (T). Moreover, the unusual pattern observed for *PRLR*-1 (T) indicates both that this allele does not contribute to the linkage signal and that including families segregating the *PRLR*-1 allele adds sufficient heterogeneity to eliminate the linkage signal. Additional analyses (see supplemental information in the Online Repository at www.jacionline.org) of these partitions and those of all possible allelic combinations demonstrate that the increased and decreased LR χ^2 values are not solely a result of increased or decreased power of the test caused by changes in the number of affected individuals included in each analysis. That is, precipitous drops in the LR χ^2 value observed for *PTGER4*, as an example, from >8 to <2 is capturing significantly more information than just the decrease in affected individuals from 154 to 86, respectively. Moreover, when only BHR individuals with the associated alleles at all 3 loci, D5S1470 (177-bp), *LIFR*-4 (T), and *PTGER4*-1 (T), were considered affected, the LR χ^2 was greater than the 99th percentile expected for its sample size, and when these same individuals were changed to unknown status, the LR χ^2 was lower than the 99th percentile expected for its sample size (see this article's Table E2 and Fig E2 in the Online Repository at www.jacionline.org). Taken together, these results suggest that nearly all of the linkage signal at D5S1470 was generated by individuals with the associated alleles at D5S1470 (as a surrogate for *ZFR* and *NPR3*), *LIFR*, and *PTGER4*. Because variation in *ZFR* and *NPR3* is associated with asthma/BHR but variation in *LIFR* and *PTGER4* is associated with asthma symptoms (but not BHR), these results further suggest that individuals with both components of the asthma phenotype (BHR and symptoms) contributed to our original linkage.

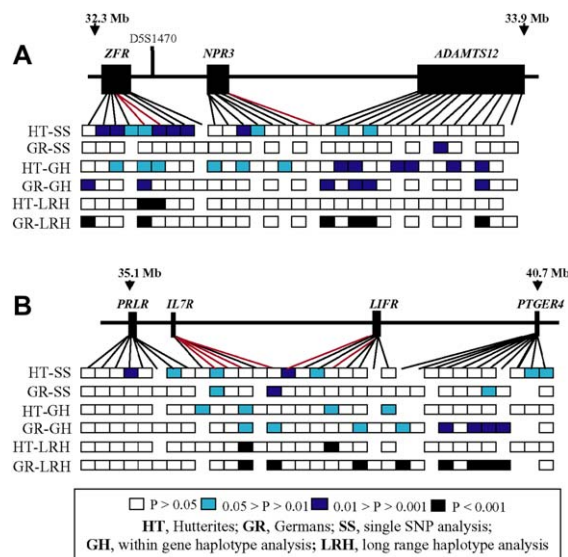


FIG 1. Relative location of the associated genes on 5p13.1 in the distal region (A) and the proximal region (B). Each typed SNP in the listed genes is pictured by squares or, if the respective SNP is typed only in 1 population, by a gap in the corresponding population. Nonsynonymous SNPs are shown by red lines. The most significant result for each SNP (with respect to phenotype) is shown. The significant results ($P < .05$) are with asthma for SNPs in *ZFR*, for BHR with SNPs in *NPR3* and *ADAMTS12*, and with asthma symptoms for *PRLR*, *IL7R*, *LIFR*, and *PTGER4*. All results in the Germans are with the asthma phenotype.

Studies in the Freiburg sample

Sixty-five SNPs were genotyped in the Freiburg sample (see this article's Table E1 in the Online Repository at www.jacionline.org). All were in HWE in the controls. However, 3 SNPs showed departures from HWE in the cases: a deficit of heterozygotes was observed for *ZFR*-9 ($P = .001$) and *ADAMTS12*-15 ($P = .004$), and an excess of heterozygotes was observed for *LIFR*-3 ($P = .003$). Because the cases and controls were genotyped at the same time, using the same method, and blind with respect to disease status, we do not believe that these deviations were caused by genotyping error and included them in our studies.

One SNP each in *ADAMTS12* and *PTGER4* and 2 SNPs in *IL7R* showed evidence of association with asthma in the Freiburg sample (Fig 1). The odds ratio of the associated genotype at each SNP was 2.55 (95% CI, 1.00-6.51; $P = .044$) for *ADAMTS12*- rs4866362, 2.43 (95% CI, 1.35-4.36; $P = .0025$) for *IL7R*- rs2289779, 1.51 (95% CI, 1.044-2.18; $P = .028$) for *IL7R*- rs1444888, and 2.06 (95% CI, 1.24-3.44; $P = .0048$) for *PTGER4*- rs1494558. Only 1 of these associated SNPs was the same SNP associated in the Hutterites (*IL7R*- rs1494558). We next constructed haplotypes composed of SNPs within each of the 7 genes associated in the Hutterites and performed association tests with each gene in the Freiburg sample. Haplotype frequencies differed significantly between asthma cases and controls for 5 of the 7 genes: 2-SNP haplotypes in *ZFR* ($P = .009$), 4-SNP haplotypes in

TABLE I. Partitioning the evidence for linkage using single polymorphisms

SNP used to partition	Included†			Excluded‡		
	χ^2	θ	N _{aff} §	χ^2	θ	N _{aff} §
Baseline*	8.147		154			
D5S1470 (117bp)	0.751	0.084	36	4.933	0.001	118
ADAMTS12-3 (T)	1.260	0.120	53	4.807	0.001	101
PRLR-1 (T)	0.000	0.250	44	9.646	0.001	110
LIFR-4 (T)	5.240	0.046	72	5.165	0.001	82
PTGER4-1 (T)	4.469	0.059	68	1.420	0.001	86

*Baseline, LR χ^2 results treating all 154 BHR individuals as affected, before partitioning.

†“Included” refers to data partitions that considers BHR individuals with the particular allele of interest as affected, and the remaining BHR individuals (those without the allele of interest) as unknown affection status.

‡“Excluded” refers to the complementary group of the data partition in which the BHR individuals with the particular allele of interest are changed to unknown affection status, and the remaining BHR individuals (those without the allele of interest) are considered affected.

§N_{aff} is the number of individuals treated as affected in the analysis.

ADAMTS12 ($P = .003$), 2-SNP haplotypes in IL7R ($P = .01$), 2-SNP haplotypes in LIFR ($P = .03$), and 4-SNP haplotypes in PTGER4 ($P = .001$) were associated with asthma. Long range multilocus haplotypes composed of SNPs in these 5 genes also revealed significant differences between asthma cases and controls for an extended 14 SNP haplotype consisting of ZFR-1, ZFR-9, ADAMTS12-18, ADAMTS12-2, ADAMTS12-4, ADAMTS12-6, IL7R-9, IL7R-2, LIFR-6, LIFR-9, PTGER4-7, PTGER4-12, PTGER4-3, and PTGER4-10 ($P < .001$). The LD structure in the German asthma cases (see this article's Fig E1, B in the Online Repository at www.jacionline.org) and controls (not shown) was similar to that observed in the Hutterites, with virtually no LD between SNPs in the distal (ZFR, ADAMTS12) and proximal (IL7R, LIFR, PTGER4) genes, suggesting the presence of multiple susceptibility loci in the 5p13 region.

DISCUSSION

Previous linkage studies identified an asthma susceptibility locus on chromosome 5p13 in the Hutterites¹⁸ and in outbred populations¹⁹⁻²² in a broad region flanking D5S1470. The fine mapping studies presented here indicate that this region harbors at least 2 loci that influence susceptibility to asthma, BHR, or asthma symptoms in the Hutterites and to asthma in outbred Germans. At least 1 locus is close to D5S1470, and may be ZFR, NPR3, ADAMTS12, or some combination of these genes, and at least 1 additional locus is more proximal, and may be IL7R, LIFR, PTGER4, or some combination of these genes.

There are multiple lines of evidence that support these conclusions. First, SNPs in ZFR, NPR3, and ADAMTS12 were primarily associated with BHR, whereas SNPs in PRLR, IL7R, LIFR, and PTGER4 were primarily associated with asthma symptoms in the Hutterites. This suggests that genes in each region influence different asthma-associated phenotypes. Second, nearly the entire linkage signal at D5S1470 in the Hutterites was generated by individuals with BHR who had the risk-associated alleles in D4S1470 (as a surrogate for ZFR and NPR3),

LIFR, and PTGER4 (see this article's Fig E2, point 6, in the Online Repository at www.jacionline.org). Thus, genes in both regions generated the original evidence for linkage. Third, associations with long-range haplotypes composed of SNPs at ZFR, IL7R, and LIFR were present in the Hutterites. The latter observation together with the lack of LD between these regions indicates that associations with alleles in these 2 regions are not likely a result of LD with a common susceptibility variant. Rather, we interpret this as evidence for the presence of more than 1 susceptibility locus. Last, associations with variation in ADAMTS12, IL7R, and PTGER4 were also associated with asthma in the outbred Germans, and the frequencies of long range haplotypes composed of SNPs at ZFR, ADAMTS12, IL7R, LIFR, and PTGER4 were significantly different between asthma cases and controls. Similar to the Hutterites, there was little LD between alleles in these 2 regions, indicating that the associations with loci in the proximal (IL7R, LIFR, PTGER4) and distal (ZFR, ADAMTS12) regions are not a result of LD with a common disease variant. The association with a long-range haplotype composed of SNPs that show little LD in both the Hutterites and Germans further suggests that genes in each region contribute to susceptibility.

Identifying susceptibility genes for complex disorders has been challenging and replicating associations in independent samples difficult. It has been suggested that regions that provide sufficient evidence for linkage in multiple populations may in fact house more than 1 susceptibility locus, because few genes will have sufficient independent effects to generate significant linkage signals.²⁸ This seems likely for asthma and atopy loci, because several of the well replicated linkages occur in regions that house many genes that have been associated with these phenotypes. These include the broad linkage region on 5q31-32 housing the T_H2 cytokine cluster as well as many other outstanding candidates (for example, see references³⁹⁻⁴²), the MHC region on 6p21 (for example, see references^{16,43,44}), and the region on 12q that includes at least 2 associated regions.^{45,46} Moreover, the first asthma/BHR gene that was identified by positional cloning, a disintegrin and metalloproteinase domain

33 (*ADAM33*) on 20p13, did not account for the entire linkage signal,¹² raising the possibility that a second gene resides in the region. This is also true of positionally cloned genes for other diseases, such as *NOD2* on 16q12 in Crohn disease⁴⁷ and *G72/G30* on 13q33 in schizophrenia.⁴⁸ Thus, our finding of more than 1 susceptibility locus on 5p may not be unusual but rather an expected finding for complex diseases such as asthma.

Although we could not localize the functional variation or the specific genes in this study, we identified 6 potential asthma and BHR susceptibility genes on 5p: *ZFR*, *NPR3*, *ADAMTS12*, *IL7R*, *LIFR*, and *PTGER4*. In the distal region, *ZFR* is the human homologue of the mouse zinc finger binding protein, which is expressed in brain and in lung. *NPR3* belongs to the family of natriuretic peptides that are involved in vascular, renal, and endocrine effects and is expressed in human placenta, adult and fetal kidney, and fetal heart. *ADAMTS12* (a disintegrin and metalloproteinase domain with thrombospondin type 1 motif) is a member of the ADAM gene family that is expressed only in fetal lung and tumor cells. Another member of this family, *ADAM33*, was recently positionally cloned as a BHR gene on 20p. Although variation in all 3 genes was associated with BHR in the Hutterites, only variation in *ZFR* and *ADAMTS12* was associated with asthma in the Freiburg sample, in which haplotypes composed of SNPs within each of these 2 genes were associated with asthma ($P = .009$ and $.003$, respectively). On the basis of this result and their expression patterns, we consider 1 or both of these genes to be the most likely candidates in the distal region. However, because of the LD between SNPs in these genes in the Hutterites and because haplotype analysis in the Germans implicated both genes, we can not narrow the association to just one of them, or rule out that SNPs in other nearby genes that are in LD with the associated SNPs in *ZFR* or *ADAMTS12* are the susceptibility variants in the distal region on 5p.

In the proximal region, 3 genes are also likely candidates. The IL-7 receptor is a member of a receptor family that includes the IL-4 receptor and exists in both membrane-bound and soluble forms. IL-7 is involved in the regulation of lymphopoiesis, and the response of cells to IL-7 is dependent on the presence of its receptor. Both IL-7 and its receptor are involved in the proliferation and differentiation of a variety of cells. The leukemia inhibitory factor receptor is a member of a cytokine receptor family that includes components of the receptors for the majority of hematopoietic cytokines and for cytokines affecting other systems. Leukemia inhibitory factor and its receptor are widely expressed in different pulmonary cell types and may play an important role in local inflammatory and neuroimmune processes.^{49,50} The prostaglandin E (EP) receptor 4 is expressed in a variety of tissues including lung, peripheral blood lymphocytes, and vasculature. Prostaglandins, along with thromboxanes and leukotrienes, constitute a group of arachidonic acid-derived compounds with biologic activities that suggest important roles in inflammation and immediate hypersensitivity. EP(2) can have proinflammatory or anti-

inflammatory effects and can modulate inflammatory reactions via the EP(2) or EP(4) receptors.⁵¹ Macrophages in sputum cells, which are significantly more abundant in patients with asthma compared with controls, express all 4 EP receptors.⁵² Thus, all 3 are excellent candidates for genes that influence asthma pathogenesis. The fact that there is also little LD between *IL7R*, *LIFR*, and *PTGER4* raises the possibility that all 3 are susceptibility genes.

In conclusion, the results of our studies indicate that the region on 5p13 harbors at least 2 asthma susceptibility loci. Six positional candidate genes have been identified, of which 5 are also outstanding functional candidates for asthma susceptibility genes. Studies in additional populations are underway to identify the specific genes and the variation in each that contributes to disease risk.

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