Chromosome 12q harbors multiple genetic loci related to asthma and asthma-related phenotypes

Benjamin A. Raby¹, *, Edwin K. Silverman¹, Ross Lazarus¹,², Christoph Lange³, David J. Kwiatkowski⁴ and Scott T. Weiss¹,⁵

¹Channing Laboratory, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA, ²School of Public Health, University of Sydney, Australia, ³Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA, ⁴Hematology Division, Brigham and Women’s Hospital, Boston, MA, USA and ⁵Harvard Partners Center for Genetics and Genomics, Boston, MA, USA

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Chromosome 12q13–24 is among the regions most frequently identified in genome-wide surveys for asthma susceptibility loci, with reports of two distinct clusters of positive linkage signals: one near the interferon gamma locus, the other near the nitric oxide synthase 1 locus. These results suggest that 12q harbors several asthma susceptibility loci. We evaluated this possibility in a subset of families ascertained through the Childhood Asthma Management Program (CAMP) Genetics Ancillary Study. Fifty-five nuclear families with at least two asthmatic siblings (212 individuals) were genotyped using 32 microsatellite markers. Non-parametric linkage analysis was performed for the asthma phenotype (qualitative). Multipoint variance component-based linkage analysis was performed for five quantitative asthma-related traits: (i) percent predicted forced expiratory volume in one second (FEV₁); (ii) dose of methacholine resulting in 20% fall in FEV₁ from baseline (PC₂₀); (iii) post-bronchodilator percent change in FEV₁ (BDPR); (iv) serum eosinophil levels (EOS); and (v) total serum IgE levels (IgE). Three separate and distinct loci demonstrated evidence suggestive of linkage: asthma at 68cM (exact \( P \)-value = 0.05), airways responsiveness (PC₂₀) at 147cM (\( P = 0.01 \)), and indices of pulmonary function (FEV₁, BDPR) at 134cM (\( P = 0.05 \) and \( P < 0.01 \), respectively). No linkage was observed for the atopy-related phenotypes. We provide further evidence supporting the presence of an asthma susceptibility locus at the proximal end of chromosome 12q, as well as new evidence for additional loci more distally that account for unique features of the asthma phenotype. Fine mapping efforts for these loci are warranted.

INTRODUCTION

There is substantial evidence that asthma is a complex hereditary disorder and that distinct asthma susceptibility genomic loci can be identified by positional cloning methods (1). Using cohorts of families with asthmatic and/or atopic members, complete data for 13 genome-wide surveys for asthma susceptibility loci have been published, identifying at least 20 distinct chromosomal regions with evidence for linkage to asthma or related intermediate phenotypes (2). Among the most frequently identified genomic regions linked to asthma is the long arm of chromosome 12 (12q13–24) (3). Of the eight genome scans published to date that have specifically evaluated a dichotomous asthma phenotype, six report evidence for linkage of chromosome 12q and asthma-related phenotypes (4–9). Focused regional surveys of chromosome 12q by four additional groups have confirmed these findings (10–13), making chromosome 12q one of the most reproducible genomic regions demonstrating linkage with asthma. Furthermore, a narrow region on mouse chromosome 10, syntenic to human chromosome 12q21.1–24.22, has been linked to airways responsiveness in quantitative trait locus mapping using an ova-sensitized mouse model of asthma (14). These data suggest that the long arm of chromosome 12q harbors at least one asthma susceptibility locus, common to many diverse populations.

Table 1 summarizes the results of the genome-wide and regional surveys demonstrating linkage for asthma to chromosome 12q. As can be seen, the linkage signals are broad and seem to cluster at the two ends of the chromosome arm: one
cluster is proximal, around the interferon-γ (IFNG) locus (65–95 cM); the other is more distal, centered on the nitric oxide synthase 1 (NOS1) locus (110–140 cM), suggesting that more than one asthma susceptibility locus exists on chromosome 12q. In our initial efforts to identify an asthma susceptibility gene on chromosome 12q, we have performed non-parametric linkage analysis (using an affected sib-pair approach) on 55 nuclear families with asthmatic sib-pairs ascertained through the Childhood Asthma Management Program (CAMP) and have replicated evidence for linkage to asthma in this cohort. In addition, using variance components quantitative trait analysis, we have observed evidence of additional intermediate asthma-related phenotypes that also map to this region, although at loci different to that linked to asthma. These results and a review of the literature are presented here.

RESULTS

Fifty-five families with at least two asthmatic offspring were available for genotyping in the CAMP cohort (212 individuals). Baseline characteristics of the 112 asthmatic siblings are presented in Table 2. This subset was similar to the remainder of the CAMP cohort (15).

In our initial screen, 22 markers mapping to chromosome 12q were genotyped, with an average intermarker density of 5.35 cM (Set A—Table 3). Using PEDCHECK, we identified 15 family marker units with genotype patterns that were inconsistent with Mendelian inheritance (1.2% of data set). These units were considered to result from genotyping errors and were discarded from subsequent analysis. On average, genotype completion rate per marker was 92.5%. There was no evidence of non-paternity or systematic genotyping error. The average allele number per marker was 11 (range 5–24). The average marker heterozygosity and single point peak information content were 0.75 and 0.72, respectively.

The results of multipoint non-parametric linkage analysis with the asthma phenotype are shown in Figure 1. Peak evidence for linkage with the asthma phenotype was observed at marker D12S368 at 65.96 cM from pter (Z-score = 1.92, LOD = 1.87, P = 0.002). A support interval defined by the one-point fall in the Z-score extended from ~61 to 81 cM. This peak spans over regions identified in three of 11 published genetic studies that have demonstrated evidence suggestive of linkage to chromosome 12q and asthma (5,10,11), and also overlaps with the 1-LOD support intervals of linkage to asthma identified in three additional studies (4,7,8), supporting the notion that an asthma susceptibility locus exists at the centromeric end of chromosome 12q.

In an attempt to further localize the asthma linkage signal in these families, 10 additional markers were genotyped, resulting in an average intermarker distance of 2.5 cM across the proximal region of chromosome 12q (56–104 cM, Marker Set B—Table 3). Repeat linkage analysis including these markers again demonstrated evidence for linkage with the asthma phenotype at the proximal region of chromosome 12q, with maximal evidence for linkage at marker D12S398 at 68.16 cM (Z-score = 1.57, LOD = 1.92, P = 0.001). However, the addition of these 10 markers did not narrow the one-point support interval (data not shown).

Linkage analysis using variance components was performed for five asthma-associated quantitative traits: (i) post-bronchodilator
FEV₁ (percent predicted); (ii) log-transformed PC₂₀; (iii) post-bronchodilator percent change in FEV₁ (BDPR); (iv) log-transformed serum eosinophil levels (EOS); and (v) log-transformed total serum IgE levels (IgE). Evidence for linkage at the distal end of chromosome 12q was observed for the three pulmonary function-associated phenotypes (Fig. 2). Maximal evidence for linkage to airways responsiveness was observed at 147 cM from pter (LOD score = 1.91, exact \( P < 0.01 \)). FEV₁ and bronchodilator responsiveness (two highly, but inversely, correlated traits in this data set: Pearson’s correlation coefficient of \(-0.35, P = 0.0002\)) demonstrated modest evidence for linkage at 134 cM from pter (FEV₁ LOD score = 1.09, \( P = 0.05 \); BDPR LOD score = 1.23, \( P < 0.01 \)). We found no evidence for linkage with either serum IgE levels or serum eosinophil levels. LOD scores for both of these traits were less than 0.2 (data not shown). Of note, there was no evidence for linkage with any of the intermediate phenotypes in the proximal region of chromosome 12q, where our evidence for linkage with asthma was observed.

**DISCUSSION**

We have replicated evidence for an asthma susceptibility locus at the proximal end of chromosome 12q and have demonstrated evidence for novel loci at the more distal end of the chromosome related to lung function, response to bronchodilators and airways responsiveness. Similar results in this region with quantitative traits have not been reported previously, although this region has been linked to asthma in four studies (6,8,13,16). Further investigation of this locus, including replication of these quantitative trait linkage results in independent populations, is warranted. Of interest, the NOS1 locus (linked in our study with bronchodilator response and lung function) has been implicated as a potential modifier of airway nitric oxide production in asthmatics (17), and modest associations between NOS1 genetic variants and asthma have been reported (18,19). Together with our data, these results suggest two separate sets of loci exist on chromosome 12q that regulate distinct asthma-related phenotypes: one at the distal end of the chromosome related to pulmonary function, and one more proximally that confers susceptibility to asthma through pathways unrelated to airways responsiveness or atopy. In this current analysis we were unable to replicate evidence for linkage with serum eosinophils and total serum IgE, despite the fact that total IgE has been linked to chromosome 12q in multiple studies, and that more than 80% of the participants in the CAMP study are atopic (20).

Although our results do not meet recognized criteria for genome-wide significant linkage (21), it is noteworthy that none of the groups demonstrating linkage on chromosome 12q have met that level of significance. Moreover, our evaluation was not performed in the context of a complete genome-wide linkage survey, but was limited to the long arm of chromosome 12q, with the *a priori* hypothesis that an asthma locus exists on chromosome 12q. Therefore, stringent criteria for significance may not be appropriate. Finally, thresholds for significant linkage should be viewed in the context of available sample size. Our reported \( P \)-values were obtained with 10 000 simulated data sets, suggesting that our findings are not likely due to chance. Our data further support the existence of a common asthma susceptibility locus at the proximal end of chromosome 12q.

As described above, chromosome 12q has been linked to asthma in six of eight genome scans where the asthma phenotype was explicitly assessed (6–9,22). The two studies that did not report evidence for linkage on chromosome 12q (the Icelandic and Finnish groups) both studied population isolates that likely demonstrate reduced locus heterogeneity (23,24). The asthma linkage signals are clustered at two ends of the chromosome arm: one proximal cluster (around IFNG) and one distal cluster (around NOS1). Of these two regions, the proximal region has more frequently demonstrated evidence for linkage to asthma. The Collaborative Study of the Genetics of Asthma (CSGA) reported modest evidence for linkage to asthma in this region in their Caucasian population (8). Ober *et al.* (22) showed linkage to asthma at the proximal end of chromosome 12q in a primary sample of 361 Hutterites and later demonstrated an association to asthma with a short tandem repeat marker in this region (25). They replicated this finding in a second sample of 292 Hutterites and the evidence for linkage to asthma persisted in a second generation study with additional markers of all 693 study subjects (5). A broad region of linkage on chromosome 12q with asthma, airway responsiveness and peripheral blood eosinophilia was observed in the Epidemiological Study on the Genetics and Environment of Asthma (7). The peak evidence for linkage with asthma was observed at 86 cM, adjacent to the one-point support interval for asthma in our current study.

In addition to the genome-wide scans, three additional focused regional surveys of chromosome 12q have replicated evidence for linkage to the proximal region of chromosome 12q (10–12,26), including two that demonstrated evidence for linkage with asthma in close proximity to our peak linkage. Most notably, Malerba *et al.* (10) studied 69 affected sib-pairs from northeastern Italy and found evidence of linkage with asthma at 67.67 cM, overlapping our region of linkage.
The proximal region of asthma linkage on chromosome 12q is broad, spanning ~26 cM if defined by the linkage peaks observed in the CSGA and Italian studies (8,10). The most current build of the public Human Genome (build 33) suggests that 437 genes have been mapped to this region, including many biologically plausible asthma susceptibility candidates, including IFNG. Several groups have demonstrated modest evidence of association with asthma for polymorphic non-coding elements in IFNG (27), but these results have not been replicated in populations that have previously demonstrated evidence of linkage to asthma (5,11). It is likely that, although IFNG variants may marginally influence asthma-related phenotypes, this locus is not responsible for the linkage findings outlined above.

Given the statistical power limitations of non-parametric linkage analysis to narrow linked regions to within several megabases in most complex traits (28), association-based studies and linkage disequilibrium mapping will be necessary to narrow this region and identify the asthma susceptibility locus. We are currently following up these results with a high density, single nucleotide polymorphism (SNP)-based fine mapping effort using the CAMP trios. We anticipate that through this effort we will identify an asthma locus that is common across many populations.

### MATERIALS AND METHODS

#### Study population

CAMP is a multicenter, randomized, double-masked, clinical trial designed to determine the long-term effects of three inhaled treatments for mild to moderate childhood asthma; 1041 children (32% from ethnic minority groups) participated. The primary outcome measure for the clinical trial was post-bronchodilator forced expiratory volume in one second (FEV₁) percent predicted. The design, rationale and methods of the trial have been described previously in detail (29), as have the primary results (15). A diagnosis of asthma was established on the basis of methacholine hyperreactivity (PC₂₀ ≤ 12.5 mg/ml) and one or more of the following diagnostic criteria for at least 6 months in the year prior to recruitment: (i) asthma symptoms at least twice a week; (ii) at least two uses per week of an inhaled bronchodilator; (iii) daily asthma medication. As part of the CAMP Genetics Ancillary Study, DNA was obtained from the CAMP subjects and 1518 of their parents, resulting in 681 complete parent–offspring trios and 246 parent–child pairs. Fifty-three of the CAMP families included a second affected offspring and two families included two additional affected offspring, resulting in 55 CAMP families (59 sib-ships, 102 trios, 525 pedigrees) for the analysis of asthma–genetic linkage.
212 individuals) available for genotyping and affected sib-pair linkage analysis. These 55 families are included in the analysis presented here.

**Phenotype information**

Phenotype data used for analysis here were obtained following a 28-day screening period of the clinical trial, during which time participants were only permitted to use inhaled albuterol on an as-needed basis. Spirometry was performed before and 15 min after bronchodilator (two puffs of albuterol) according to American Thoracic Society (ATS) recommendations with a volume-displacement spirometer, and airway responsiveness was assessed by methacholine challenge by the Wright nebulizer-tidal breathing technique (29). Methacholine challenge was not performed within 4 weeks of an upper respiratory tract infection or use of oral corticosteroids. Bronchodilator response was calculated using the following equation:

\[
100 \times \left( \frac{\text{post-bronchodilator FEV}_1 \text{ (L)}}{\text{pre-bronchodilator FEV}_1 \text{ (L)}} \right) / \text{pre-bronchodilator FEV}_1 \text{ (L)}.
\]

Total serum IgE was measured by radioimmunoabsorbent assays (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden) from blood samples collected during the screening sessions of the CAMP study. Total blood eosinophil counts were performed by each center using standard methods. Serum total IgE levels and eosinophil counts were log-transformed for analysis.

**DNA genotyping**

For the initial analysis, 23 short tandem repeat (STR) markers were selected from the Marshfield database (http://research.marshfieldclinic.org/genetics) to cover chromosome 12q with a marker density of \(~5\) cM (Set A—Table 3). One marker failed. The results presented here include the 22 markers successfully amplified (average density \(~5.35\) cM). Oligonucleotides with fluoropore labels were purchased from Research Genetics (Huntsville, AL). Assays were amplified in standard singleplex polymerase chain reactions (PCR) with 5 ng DNA. PCR products were pooled into groups of 6–8 and run on ABI 3100 Genetic Analyzers (Applied Biosystems, Foster CA). Allele calling employed ABI Genotyper software v3.7 (Applied Biosystems) and was verified manually. In a second round of genotyping, an additional 12 markers were added (Set B—Table 3) with the goal of increasing the marker density to 2 cM at the centromeric end of chromosome 12q; two markers did not amplify properly and therefore were not included.
Where possible, marker order and relative genetic distance were compared with the deCODE linkage map (30).

Analysis

All markers were assessed by PEDCHECK (version 1.0) (31) for pedigree inconsistencies. The software package Merlin (32) was used for multipoint non-parametric linkage analysis for the qualitative phenotype of asthma and for multipoint variance components (VC) linkage analysis of five quantitative phenotypes: (i) FEV1; (ii) dose of methacholine resulting in 20% fall in FEV1 from baseline (PC20); (iii) post-bronchodilator change in percent predicted FEV1 (BDPR); (iv) serum eosinophil levels (EOS); and (v) total serum IgE levels (IgE). PC20, EOS and IgE were normally distributed following log-transformation. A total of 10,000 simulations for each phenotype were performed using a gene-dropping simulation application (available in Merlin). The simulation replaces input data with simulated chromosomes conditional on the CAMP pedigrees family structure, the observed marker spacing, and allele frequencies, and missing data points. Results of these simulations were used to obtain exact \( P \)-values.

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