European Journal of Human Genetics (2009) 17, 636 – 643

www.nature.com/ejhg

ARTICLE

Significant evidence for linkage to chromosome 5q13 in a genome-wide scan for asthma in an extended pedigree resource

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Asthma is a multifactorial disease with undetermined genetic factors. We performed a genome-wide scan to identify predisposition loci for asthma. The asthma phenotype consisted of physician-confirmed presence or absence of asthma symptoms. We analyzed 81 extended Utah pedigrees ranging from three to six generations, including 742 affected individuals, ranging from 2 to 40 per pedigree. We performed parametric multipoint linkage analyses with dominant and recessive models. Our analysis revealed genome-wide significant evidence of linkage to region 5q13 (log of the odds ratio (LOD) = 3.8, recessive model), and suggestive evidence for linkage to region 6p21 (LOD = 2.1, dominant model). Both the 5q13 and 6p21 regions indicated in these analyses have been previously identified as regions of interest in other genome-wide scans for asthma-related phenotypes. The evidence of linkage at the 5q13 region represents the first significant evidence for linkage on a genome-wide basis for this locus. Linked pedigrees localize the region to approximately between 92.3–105.5 Mb.
European Journal of Human Genetics (2009) **17,** 636–643; doi:10.1038/ejhg.2008.236; published online 17 December 2008

Keywords: asthma; linkage analysis; gene mapping; extended pedigrees

Introduction

Asthma is a clinically heterogeneous phenotype characterized by intermittent, reversible airway constriction with varying degrees of severity, frequency and age-at-onset of symptoms observed between affected individuals.[1] Asthma is a common disorder of the airways with an estimated prevalence of 7% in the United States,[2] but it also represents a substantial global health burden with an increasing rate of incidence observed in recent decades.[3]

Although the precise etiology of asthma remains undefined, it is accepted that asthma is a multifactorial disease resulting from complex interactions between environmental and genetic risk factors.[4] Multiple lines of evidence support a genetic contribution to asthma. Concordance rates for asthma were found to be significantly higher among MZ twins than DZ twins in several studies.[5–7] Family history has been identified as a risk factor for asthma in numerous populations, with odds ratios for first-degree relatives of asthma ranging from 1.5 to 9.7.[8] Significant excess relatedness has been observed among close and distant relatives for a severe asthma

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Received 23 January 2008; revised 6 November 2008; accepted 13 November 2008; published online 17 December 2008

phenotype.[9] Despite evidence for a heritable component, segregation analyses of high-risk pedigree resources have provided inconsistent evidence for the mode of inheritance for asthma, complicating genetic investigations.[10–13]

Strong evidence for a heritable contribution to asthma has motivated 35 published genome-wide scans for predisposition loci to asthma-related phenotypes carried out in 22 distinct study populations in fourteen countries.[14–20] Genome-wide scans for asthma have resulted in over 30 suggestive or significant regions of interest in the human genome.[14] In a recent meta-analysis 372 separate gene–disease association studies were identified for asthma-related phenotypes, some of which were motivated by evidence for predisposition loci obtained from linkage analyses.[21] The large number of disease–gene association studies for asthma indicates a lack of replication between studies, and points to a complex genetic etiology for asthma, and the possibility of predisposing factors that are unique to different populations. Despite recent progress, the lack of confirmation between linked regions identified from genome-wide scans reinforces the need to pursue genetic investigations of asthma with new powerful resources and with varied approaches.

We performed a genome-wide scan for asthma susceptibility loci on 81 extended Utah pedigrees ascertained for multiple related individuals diagnosed with asthma. The use of extended pedigrees may provide increased power to detect predisposition loci because of the large number of informative meioses.[22] Probands were identified from a new large Utah genealogy linked to hospital data from the largest healthcare facility in Utah. Data collected for study subjects included prior medical history, risk factor data, family history of respiratory disorders and spirometry data. Our analysis of the Utah asthma pedigrees utilized a general asthma phenotype defined as physician confirmed presence or absence of asthma.

Subjects and methods

The Utah population has been described as being of Northern European descent, having similar inbreeding levels as other parts of the United States,[23–24] and having characteristics conducive to genetic studies including large family size and an interest in genealogy. High-risk pedigrees were ascertained through a Utah genealogy resource linked to hospital diagnosis data from Intermountain Health Care, the largest health care provider in Utah, and also through local advertisement and physician referral. These patients and their connecting relatives were contacted and sampled by the Genetic Research group at Intermountain Health Care at LDS Hospital between 1996 and 2000. Informed consent was obtained for each individual involved in the study. Institutional Review Board approval was obtained from Intermountain Health Care and the University of Utah.

Asthma affection status was determined by a comprehensive clinical evaluation. Study subjects completed a modified National Heart Lung and Blood Institute Collaborative Studies on the Genetics of Asthma questionnaire specific to asthma, and pulmonary function testing by spirometry was performed according to Intermountain Thoracic Society standards for individuals age 6 or older.[25] In some cases, diagnosis also relied on available medical records, pre- and post-bronchodilator testing, or methacholine challenge testing.[26] On the basis of the results of the clinical evaluation an experienced pulmonologist conferred a diagnosis of affected, unaffected or unclassifiable. A total of 1451 patients were seen, resulting in 744 affected individuals, 628 unaffected individuals and 79 unclassifiable individuals. The resulting diagnosis provides the basis for the phenotype definition specific to this analysis.

Study subjects provided a blood sample for DNA. Informative individuals in pedigrees with at least 2 sampled asthma cases were genotyped ($n = 1314$) on a set of 535 fluorescent dye-labeled microsatellite markers by Myriad Genetics. Markers spanned the entire genome, including the pseudoautosomal region of the X chromosome, with an average spacing between markers of 6.4 cM. The genetic map was developed internally using CRIMAP software on 3916 meioses from a set of high-risk Utah pedigrees ascertained for asthma and multiple other disorders. It corresponds closely to published deCODE maps.[27–29] Inheritances were verified using PEDCHECK software.[30] Inconsistencies were re-genotyped where possible. Unresolved inconsistencies, including Mendelian errors which occurred at a rate of 0.036%, were set to missing.

Linkage analysis was performed with MCLINK which estimates multipoint inheritance vectors using a Monte Carlo Markov Chain (MCMC) methodology employing a blocked Gibbs sampling method to infer phase.[31] The advantage of the MCMC approach is the ability to analyze entire pedigrees without constraint on size or nonunilineal structure, allowing the analysis to take advantage of all inheritance information. MCLINK utilizes the robust multipoint statistic proposed by Goring and Terwilliger (hereafter referred to as TLOD),[32] which has been implemented in MCLINK. The TLOD statistic uses multipoint inheritance vectors to estimate inheritance probabilities at specific marker position and is maximized over the recombination fraction (hence, theta-LOD, or TLOD), providing a multipoint linkage statistic that is robust to model misspecification.[33] For each marker, the heterogeneity TLOD (het-TLOD) statistic was calculated with HOMOG software to account for any interfamilial heterogeneity.[34] Unbiased marker allele frequencies were estimated from thousands of individuals in high-risk Utah pedigrees genotyped for multiple disorders.[35]

Parametric analyses were performed using general dominant and recessive models. Nonparametric methodologies are generally favored in linkage analysis of complex traits in small family structures because no assumptions about the mode of inheritance are required for these methods. However, it has been shown that in extended pedigree settings, analyses relying on non-parametric (or 'model-free') methodologies will approach similar power as parametric analyses only when stringent assumptions are in place.[36] Parametric linkage analyses utilizing general models have been shown to be effective in detecting evidence for linkage when information about the mode of inheritance is not well established.[37,38] Further, simulation has shown that in a complex disease setting, general parametric analyses are more powerful if they include both a dominant and recessive model as it is more critical to distinguish the mode of inheritance at the linked locus, and not that of the disease in general.[37] We assumed a disease allele frequency of 0.005 and 0.05 for the dominant and recessive models, respectively; both models assumed a penetrance of 50% for gene carriers and 0.5% for nongene carriers.

Significance for genome-wide linkage was evaluated according to the thresholds established by Lander and Kruglyak[39] additionally corrected for the two models analyzed. Using a previously published method, we established that the two models corresponded to 1.9 independent tests and we applied a Bonferonni correction to correct for this.[40] As a result of the correction, LOD scores in excess of 3.6 were considered significant, and LOD scores in excess of 2.1 were considered suggestive.

A 1-LOD support interval based on the TLOD statistic was used to provide general boundaries for a region of interest. However, locus heterogeneity and random noise from unlinked pedigrees in the heterogeneity-TLOD calculation can shift the support interval. To delimit the regions of interest we also used an alternative recombinant-mapping approach based on pedigrees with evidence of linkage to the region of interest.[27,41] Linked pedigrees were considered as those with a classic multipoint LOD score >0.59 (nominal $P=0.05$) within 30 cM centered on the TLOD peak. Within this region, recombination events within linked pedigrees can be estimated. A recombination event was identified by a reduction in haplotype sharing among affected individuals in the pedigree, and was defined as the outermost marker position of a 0.5 LOD unit decrease in a linked pedigree.[27] We defined a localized region defined by the linked pedigrees as delimited by the outermost of three recombination events in any linked pedigree in either direction. The linked pedigree region of interest has been described as a 99% credible interval, thereby providing greater precision for localization than a 1-LOD support interval centered on the maximal TLOD statistic.[42] A more formal description of our localization method is given by Camp *et al*.[42]

In linked pedigrees we identified all affected individuals who shared the segregating haplotype(s) that contributed to the LOD score, according to the model calculated. For pedigrees linked under the recessive model this included all affected individuals who clearly shared two of any segregating predisposition haplotypes in the pedigree. For pedigrees linked under the dominant model this included all affected individuals sharing the single segregating predisposition haplotype in the pedigree.

Results

A total of 81 informative high-risk asthma pedigrees were selected for genotyping, each with between three and six generations. A total of 1874 individuals in these pedigrees were analyzed with a range of 6–97 total individuals per pedigree. The pedigrees included 742 affected individuals (per pedigree range 2–40), 624 unaffected individuals and 508 unknown individuals. Individuals with unknown disease status or with no genotype data were included in the analysis to preserve the structure of the pedigrees. Of the entire resource, 1314 informative individuals were genotyped (70%), 693 of whom were affected (93% of individuals with affected status). Table 1 provides a summary description of the pedigree resource.

Figure 1 contains a graph of the het-TLOD for the genome-wide scan for the primary analysis of the complete pedigree resource, showing the results of both the dominant and recessive models. After correction for multiple testing, one region exceeded the threshold for significant evidence for linkage on chromosome 5q13 at marker D5S2498 (106.6 cM from pter, het-TLOD = 3.8, nominal $P=0.000016$), using the general recessive model. One other region reached suggestive evidence. This region occurred using the dominant model on chromosome 6p21 at marker D6S1281 (44.0 cM from pter, het-TLOD = 2.1,

Table 1 Characteristics of 81 families (1874 individuals) ascertained for asthma

	All kindreds (n = 81)
Mean number of individuals/pedigree (range)	23 (6–97)
Mean number of affecteds/pedigree (range)	9 (2–40)
Mean number of typed affecteds/pedigree (range)	9 (2–36)
Median age (range)	42 (12–97)
Median age at diagnosis (range)	9 (0–79)
% indicating atopic symptoms[a]	52
Average predicted FEV_1/FVC[b] (%)	74.4
Average measured FEV_1/FVC[b] (%)	83.1
% indicating history of smoking[a]	13
% Caucasion[a]	99

Abbreviations: FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.
[a]As per questionnaire.
[b]Predictions derived from sex, height and weight.

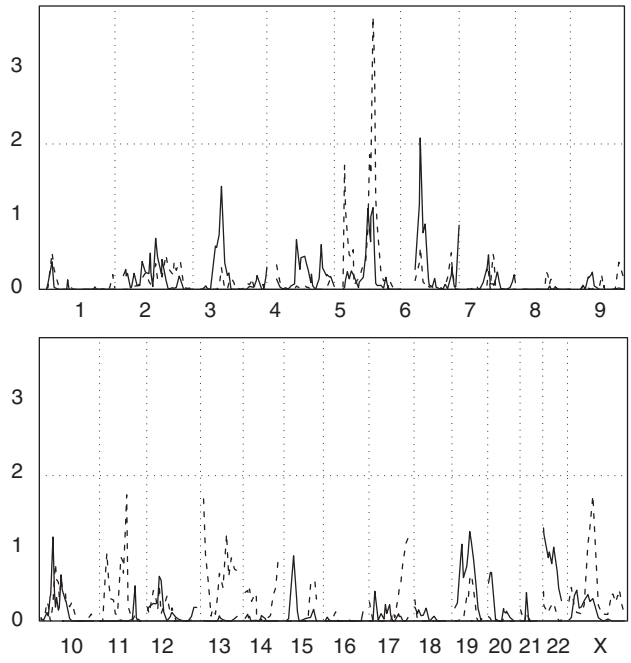

Figure 1 Results of genome-wide scan for asthma, het-TLOD plotted, *x* axis represents the chromosome. The solid line depicts results from the dominant model and the dashed line depicts the recessive model.

Table 2 Markers with TLOD scores greater than 1.0 in genome-wide scan using a general dominant or recessive model

Chromosome	*Mb position*	*Best marker*	*TLOD score*	*Model*
3	58.9	D3S1766	1.4	Dom
5	92.2	D5S2498	3.8	Rec
6	25.4	D6S1281	2.1	Dom
10	16.6	D10S674	1.2	Dom
11	75.9	D11S1366x1	1.7	Rec
13	20.4	D13S175x1	1.7	Rec
17	74.6	D17S928x1	1.1	Rec
19	42.5	D19S713	1.2	Dom
22	18.8	GATA198B05	1.3	Dom
X	50.2	DXS8023	1.7	Rec

nominal $P = 0.00098$). Table 2 shows all regions with heterogeneity-TLOD scores which exceeded 1.0.

The 1-LOD support interval for the significant region on chromosome 5q13 was 16 cM in size, located between markers D5S1347 and D5S1453 (at approximately 98.8 and 115.4 cM, respectively). Using the linked-pedigree localization method, 16 pedigrees were considered linked to the region (5 pedigrees with a classic multipoint LOD score between 0.59 and 1.0, 8 between 1.0 and 1.5, and 3 with LOD score greater than 1.5). Figure 2 shows a recombination map for the 16 linked pedigrees, ordered by LOD score, displaying the region estimated to be shared by cases in each pedigree in Mb position. No conflicts are identified in the region. The three closest recombinant events to the left and right of the region shared by all linked pedigrees were used to define a localized region between 92.3 and 105.5 Mb (13.2 Mb in length and approximately 8 cM between 106.6 and 115.4 cM) which is contained within the 1-LOD support interval. Considering the 16 pedigrees linked to this region, 88 of the 168 affected individuals could be clearly identified as sharing two of any of the segregating predisposition haplotypes in the pedigree (enumerated by pedigree in Figure 2).

The 1-LOD support interval for the suggestive region on chromosome 6p21 was 26 cM in size, located between markers D6S1006 and D6S2417 (at approximately 27.2 and 53.2 cM, respectively). Using the linked-pedigree localization method, 7 pedigrees were identified as linked (4 pedigrees with a classic multipoint LOD score between 0.59 and 1, 1 between 1 and 1.5 and 2 with LOD score greater than 1.5). Figure 3 shows a recombination map for the 7 linked pedigrees, ordered by LOD score, displaying the region estimated to be shared by cases in each pedigree in Mb position. No conflicts were identified in the region. Using three recombinant events on each side, the region was localized as between 28.7 and 48.9 Mb (20.2 Mb in length and approximately 24.3 cM between 44.7 and 69 cM). Recombination events within linked pedigrees indicate that a predisposition locus in this region may be as much as 9 cM centromeric of the peak het-TLOD. Within the 7 pedigrees linked to this region, 35 of the 45 affected individuals were clearly identified as sharing the hypothesized segregating predisposition haplotype (listed by pedigree in Figure 3).

We did not identify any statistically significant phenotypic differences between members of linked and unlinked pedigrees nor between affected and unaffected individuals within pedigrees linked to the two regions of interest (5q13 and 6p21) with respect to spirometry measures or questionnaire data.

Discussion

Our analysis provides significant evidence for linkage to a general asthma phenotype on chromosome arm 5q, with 16 out of 81 pedigrees linked in this region. The best linkage evidence for this region occurred with a recessive model, and at least 88 of 168 affected individuals clearly shared two of any hypothesized segregating predisposition haplotypes in the 16 linked pedigrees. For many affected individuals, there was insufficient genetic data to determine the sharing. However, although this region was identified using a recessive model, these are observational findings and the true underlying mode of inheritance may differ from this simple model. While the linkage evidence for chromosome region 5q13 represents the first significant finding for this region, this region has been previously

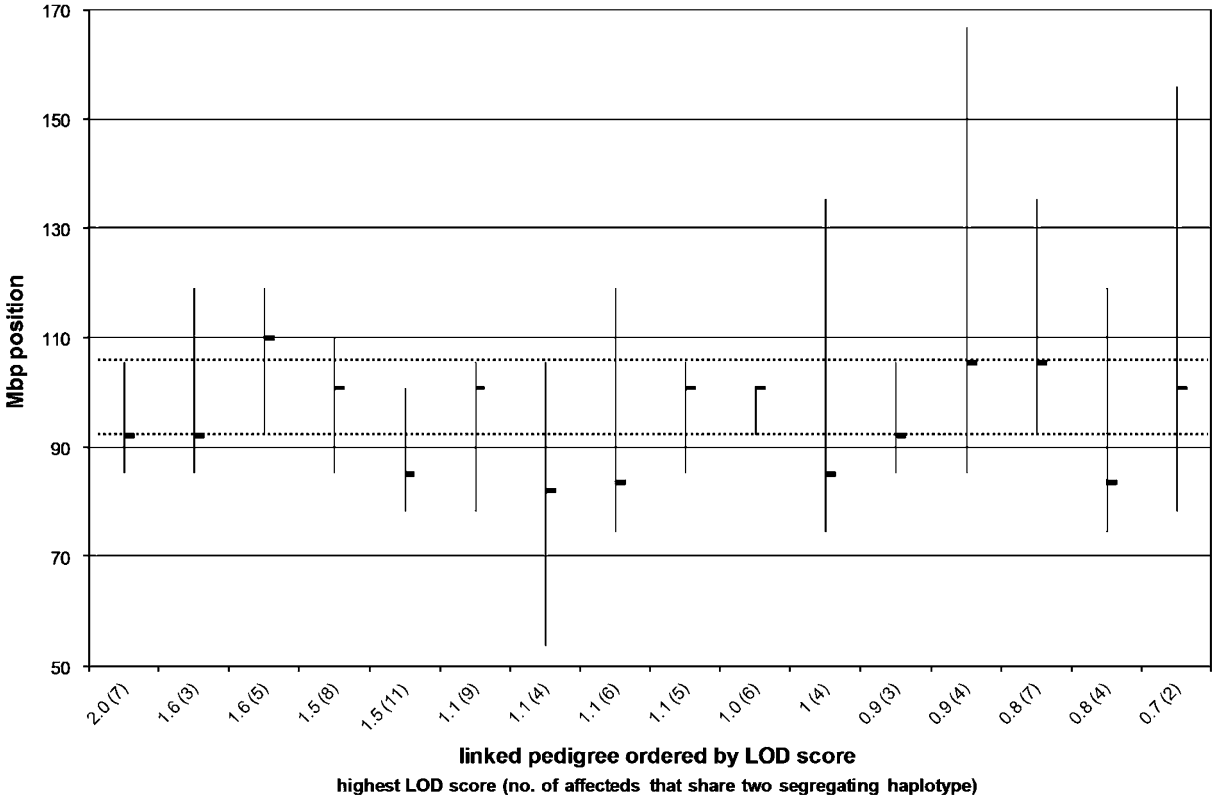

Figure 2 Shared linked-haplotype segments as delimited by estimated recombination events plotted against bp position for each of the 16 pedigrees linked to chromosome 5q13 region, ordered by maximum by-pedigree LOD score in this region. The three-recombinant boundaries are depicted by the horizontal dashed lines. The locations of the maximum by-pedigree LOD scores are shown as horizontal ticks along the plotted vertical line for each pedigree. The value of the maximum LOD score for each pedigree is given below the plot. The numbers in parentheses indicate the number of affected individuals in each pedigree who could clearly be determined to share two of the hypothesized segregating predisposition haplotypes, and the total number of affected individuals.

suggested as of interest in several genome-wide scans for asthma.[14,15,43,44] We report all findings within 25 cM, because the position of a linkage peak may be shifted from the underlying predisposition locus due to noise from unlinked pedigrees. Ober *et al*[44] were the first to report evidence for linkage ($P = 0.007$, equivalent LOD = 1.3) to marker D5S1462 using a general asthma phenotype definition in a founder population. The marker D5S1462 occurs within 1 cM of our reported linkage peak for 5q13. Haagerup *et al*[43] reported linkage to marker D5S1466 (LOD = 2.03), which occurs 12 cM telomeric of our linkage peak, using a phenotype based on clinical asthma diagnosis. Separately, Bouzigon *et al*[15] reported a LOD score of 1.87 at marker D5S424 (approximately 23 cM centromeric from our linkage peak) using a quantitative trait loci phenotype based on a scoring mechanism that included several clinical aspects of asthma diagnosis. Ferreira *et al*[14] also reported linkage to marker D5S424 (LOD = 2.7) using a phenotype definition based on pulmonary function. Comparison of the mode of inheritance between these studies is not possible since the previous studies relied on nonparametric methods. Potential candidate genes within the localized region of chromosome 5q13 may include ARSK1, ARTS1, PCSK1, LRAP and CAST. The candidate gene CRHBP proposed by Bouzigon *et al*[15] is approximately 20 Mb centromeric of our region of interest on chromosome 5. It should be noted that previous studies have reported significant evidence for linkage of asthma phenotypes to a nearby chromosome 5q31–33 region, but that we consider this region to be unrelated to our finding since the peak linkage signals in these studies were at least 35 cM telomeric of the marker where our linkage peak occurred.[45–48] In addition, a recent genome-wide association study for asthma identified the single nucleotide polymorphism marker rs10476658 (approximately 2 cM telomeric of our linkage peak) as exceeding the 5% false discovery rate for that study.[49]

Four previous studies reported moderate evidence for linkage within 5 cM of the peak for the suggestive region on chromosome 6p21.[46,50–52] In addition, the positionally cloned HLA-G occurs in this region and risk-conferring alleles have been identified for asthma in a predominantly

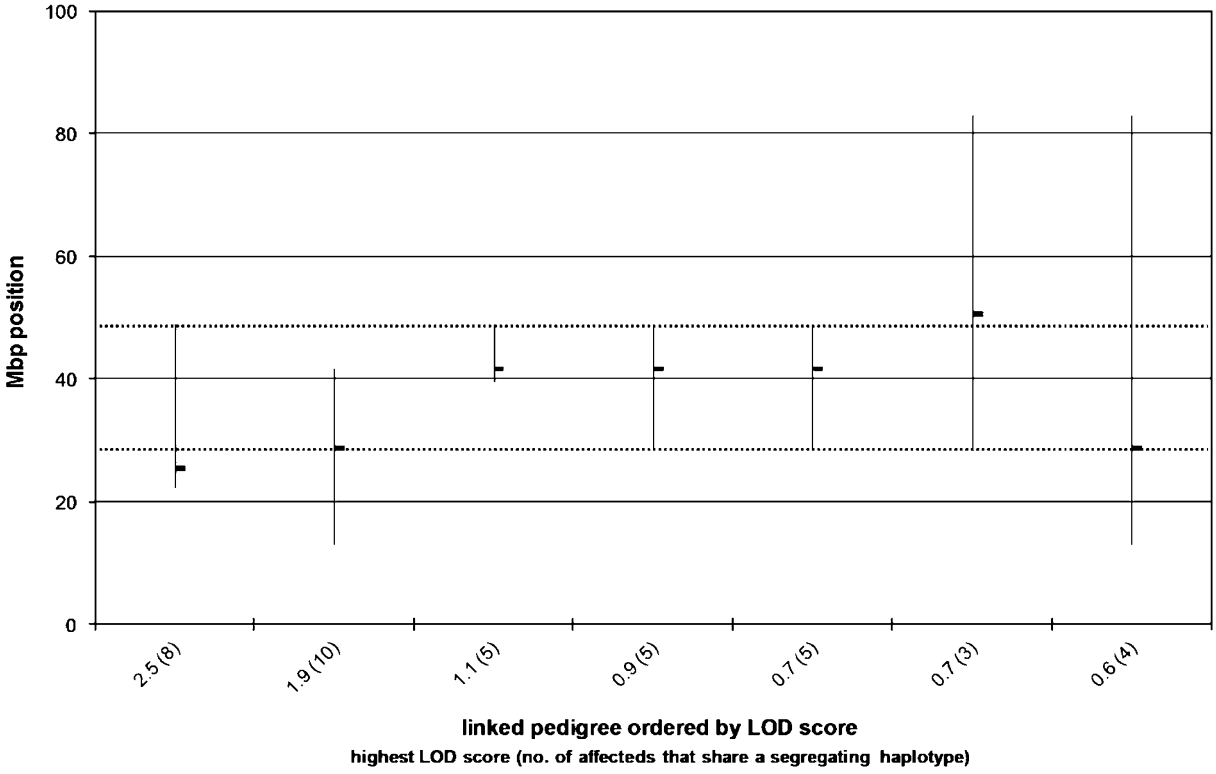

Figure 3 Shared linked-haplotype segments as delimited by estimated recombination events plotted against bp position for each of the seven pedigrees linked to chromosome 6p21 region, ordered by maximum by-pedigree LOD score in this region. The three-recombinant boundaries are depicted by the dashed lines. The locations of the maximum by-pedigree LOD scores are shown as horizontal ticks along the plotted regions for each pedigree. The value of the maximum LOD score for each pedigree is given below the plot. The numbers in parentheses indicate the number of affected individuals in each pedigree who share the hypothesized segregating predisposition haplotype, and the total number of affected individuals.

Caucasian study cohort.[53,54] Future plans include the investigation of segregation of previously identified risk-conferring alleles through the pedigrees that are linked to this locus. The consistency of findings between our results and those of other studies reduces the possibility that we have observed false positive linkage signals for the regions of interest we report.

Our analysis of extended pedigrees failed to confirm linkage to several regions of interest reported in previous genome-wide scans for asthma such as regions on chromosomes 2, 3, 4 and 12.[14] It is possible that the lack of confirmation for regions on these chromosomes is a result of intrafamilial heterogeneity within our extended pedigrees that may occur if the frequency of the susceptibility allele is common, from our use of a general phenotype definition that may be less powerful in the presence of phenotypic heterogeneity, or because of potential differences in the genetic backgrounds of different populations studied in previous linkage and association reports.

The primary strength of our approach is the use of extended high-risk pedigrees, as these can provide information about shared segregating haplotypes and localization through the observation of recombination events within individually powerful pedigrees. The extended pedigree resource was selected for high risk for asthma which may reduce the chance of sporadic cases in the pedigrees.

The results of our analysis provide the first genome-wide significant linkage evidence for 5q13 as an asthma predisposition locus. Our localization efforts indicate the predisposition variant(s) reside between 92.3 and 105.5 Mb. Our evidence for 6p21 and 4q21 confirmed previously identified regions of interest in genome-wide scans for asthma related-traits and support the hypotheses that predisposition loci for asthma are likely to exist in these regions as well.

Acknowledgements

Special thanks to the family members who participated in this research and to the study coordinators who worked with them to create the family resource, including: Denise Daley, Tracey Butcher, Greggory Wood, Ryan Huffman, Brian Chase, and to applied biostatistician James Farnham. CT was supported by a National Library of Medicine Training Grant to the University of Utah Department of Biomedical Informatics, 1T15LM007124. This work was in part supported by CA098364 (to NJC). The original pedigree collection, phenotyping and genotyping activities were funded by a grant (to LCA) through Intermountain Healthcare from Myriad Genetics (1995–2000).

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