

Short communication

Evidence for an asthma risk locus on chromosome Xp:
a replication linkage study

Background: Asthma is a complex genetic disorder characterized by chronic inflammation in the airways. Identification of genetic risk factors for asthma has been complicated due to genetic heterogeneity and influence from environmental risk factors. Despite the fact that multiple genetic linkage studies have been carried out the results are still conflicting and call for replication experiments. A Danish genome-wide scan has prior reported evidence for candidate regions for asthma susceptibility genes on chromosomes 1p, 5q, 6p, 12q and Xp. Linkage to chromosome 12q was later confirmed in the same replication sample as used in the present study. The aim of the study was to replicate linkage to candidate regions for asthma in an independent Danish sample.

Methods: We performed a replication study investigating linkage to candidate regions for asthma on chromosomes 1p36.31-p36.21, 5q15-q23.2, 6p24.3-p22.3, and Xp22.31-p11.4 using additional markers in an independent set of 136 Danish asthmatic sib pair families.

Results: Nonparametric multipoint linkage analyses yielded suggestive evidence for linkage to asthma on chromosome Xp21.2 (MLS 2.92) but failed to replicate linkage to chromosomes 1p36.31-p36.21, 5q15-q23.2 and 6p24.3-p22.3.

Conclusions: The replication results provide evidence for chromosome Xp21 to harbour a susceptibility gene for asthma in the Danish population. To our knowledge, the study is the first to replicate evidence for linkage to chromosome X. A susceptibility gene for asthma on chromosome X could potentially explain observed gender differences in asthma prevalence.

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Asthma [OMIM 600807] affects an estimated 155 million individuals in the world and is the most common chronic disease of childhood in developed countries. Asthma is a complex disorder and twin and family studies have suggested a significant genetic component, with heritability estimates ranging from 0.4 to 0.8 (1). However, numerous environmental factors have also been found to be involved in the disease risk affecting genetically susceptible individuals (2).

By performing family linkage studies, new genes for asthma can be identified as the method needs no *a priori* knowledge on mode of inheritance or possible molecular disease mechanisms. The ultimate goals for identification of asthma risk genes are to be able to identify genetically susceptible individuals and to develop individualized medicine based on genotype.

Multiple genome-wide linkage screens for asthma and atopy have been carried out during more than a decade

showing very variable results (3). The differences in results could be due to differences in phenotype definitions as well as genetic heterogeneity, confounding effects of environmental risk factors and differences in study design and populations as well as reported false positive results and lack of power in replication studies. Several chromosomal regions have been suggested and some reported by multiple groups. The most replicated regions are on chromosome 2q, 4q, 5q23-31, 6p24-21, 11q13-21, 12q21-24, 13q12-14, 16q21-23 and 19q (3). Only five regions have met the criteria of genome-wide statistical significant linkage to asthma. Significant evidence for linkage has been shown to 5q31-q33 to mite-sensitive asthma (4); 6p21 to asthma (5); 12q24.33 to asthma (6), 14q24 to asthma (7) and 20p13 to asthma and bronchial hyper-responsiveness (8).

Despite the significance of these results, few have been replicated, which must be the next step to identify the susceptibility genes.

Our group have previously reported results from a genome-wide scan for asthma in the Danish population

Abbreviations: cM, centimorgan; MLS, maximum-likelihood-score.

(9). The results were based on a limited number of families and the findings call for confirmation in independent families.

The genome-wide scan for asthma reported suggestive candidate regions on chromosome 1p36, 5q31, 6p24-22, 12q24 and Xp11 (9). Fine-scale mapping of these regions in the original sample showed linkage to 6p, 12q and Xp (10). Linkage to chromosome 12q has later been confirmed in the larger independent asthma sample used in the present study and a splice factor (*SFRS8*) was identified as the possible candidate gene from the region (6).

This study reports linkage results from fine-scale mapping in 136 sib pair families of the remaining asthma candidate regions known from the Danish genome-wide scan: 1p36.31-p36.21, 5q15-q23.2, 6p24.3-p22.3, and Xp22.31-p11.4.

Methods

Participants

The sample consisted of 136 asthma affected sibpair families, including 31 pairs of brothers, 50 pairs of sisters and 55 brother-sister pairs with asthma.

The asthma inclusion criteria were clinically based on recurrent cough, wheezing and dyspnoea and a positive methacholine inhalation challenge identical to that used in a larger Danish study (11). A positive response was equivalent to a provocative concentration causing a 20% fall in forced expiratory volume in one second (FEV_1) < 8 mg/ml. All included probands had lung function measurements including FEV_1 and forced vital capacity. Methacholine challenge was carried out in all probands unless FEV_1 < 70% predicted value or the subject refused the challenge.

Molecular genetics

Genomic DNA was prepared from whole blood by standard methods. Microsatellite markers were analysed with an average distance of 2.9 cM (range 0.54–8.46) in regions pointed out in an earlier genome-wide scan (9). The following markers were used for 1p36.31-p36.21: D1S2870, D1S1646, D1S548, D1S508, D1S2667, D1S228; for 5q14.3-q23.2: D5S1463, D5S1462, D5S1503, D5S433, D5S1466, D5S1720, D5S494, D5S1505, D5S657, GATA70D09, D5S1495; for 6p24.3-22.3: D6S1640, D6S277, D6S470, D6S429, D6S1006, D6S260, D6S274, D6S1959 and for Xp22.31-p11.4: DXS1223, DXS7108, DXS999, DXS1226, DXS989, DXS8039, DXS1214, DXS9907, DXS8090, DXS1068. Primer sequences were obtained from The Genome Database (<http://www.gdb.org>). PCR was performed using amplification conditions similar to those described at The Genome Database. The amplified products were separated using ABI377 automated DNA sequencer and analysed using ABI software (Applied Biosystems).

Statistical analysis

Data from the affected sib pair families were analysed by non-parametric multipoint linkage analysis using the maximum-likelihood-score (MLS) approach. The software MAPMAKER/SIBS (12) was used for calculation of multipoint maximum likelihood

IBD and information content. An increment step setting of five facilitated computing of MLS scores between each marker was used.

Results

We fine-mapped four chromosomal regions in a Danish asthma sib pair sample of 136 sib pair families using 35 markers with an average distance of 2.9 cM. The regions on chromosome 1p36.31-p36.21, 5q21.3-q23.2, 6p24.3-p22.3 and Xp22.31-p11.4 were previous pointed out as candidate regions (defined by a $MLS > 1.5$) for asthma in another Danish sample in a genome-wide scan (9). We were interested in replicating the original results from the genome-wide scan in another independent Danish sample ascertained due to their asthma status.

As the mode of inheritance for asthma is unknown we used a nonparametric multipoint linkage analysis searching for increased allele sharing within the affected sib pairs. We found strong evidence for linkage on chromosome Xp21 centred on marker DXS1214 revealing a peak MLS of 2.92 at the marker locus (Fig. 1). Maximum-likelihood-score above 2.5 was obtained in a region of less than 2 Mb (DXS8039–DXS9907, according to UCSC Genome Browser, assembly Mar 2006). Increased allele sharing was also observed 10 cM distal to DXS1214 around marker DXS999 (MLS 1.25). The average information content of the map based on all markers was 89% and 95% at peak MLS (Table 1).

We did not find evidence to support the linkage suggested in the genome-wide scan on chromosome 1p36.31-p36.21 in a region spanning 15.9 cM nor on chromosome 5q21.3-q23.2 in a region spanning 34 cM nor on chromosome 6p24-p21.3 in a region of 22 cM (Table 1).

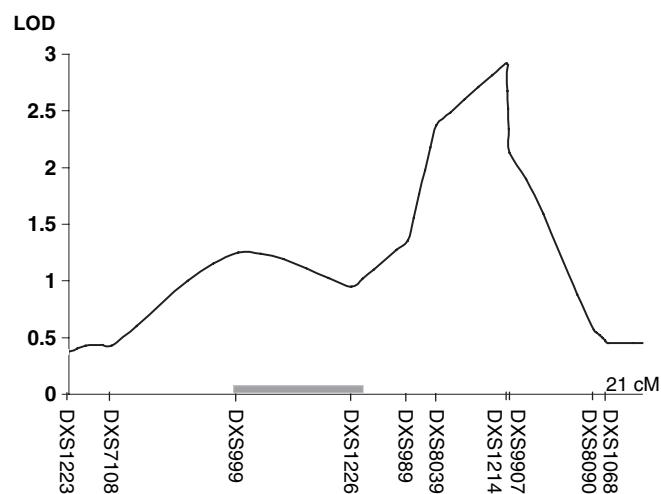


Figure 1. Maximum likelihood IBD curve on chromosome Xp. The *x*-axis represents the genetic distance in the region. The *y*-axis depicts the MAPMAKER/SIBS LOD score when using asthma as a qualitative trait. The grey bar represents 5 cM.

Table 1. Specified results from fine-scale mapping of four candidate regions

Cytogenetic position	Marker map positions (cM)	Marker	Info	MLS – replication study	MLS – original genome scan (9)	MLS – original sample after finemapping (10)
Xp22.13	23.26	DXS999	0.91	1.25		1.25
Xp22.11	27.59	DXS1226	0.94	0.95		
Xp22.11	29.76	DXS989	0.97	1.35		
Xp21.2	30.84	DXS8039	0.92	2.37		2.11
Xp21.2	33.54	DXS1214	0.95	2.92		1.85
Xp21.1	33.54	DXS9907	0.95	2.13	1.66	1.87
1p36.31	14.59	D1S1646	0.97	0.04	1.39	
1p36.23	16.22	D1S508	0.96	0	2.02'	
1p36.22	24.68	D1S2667	0.9	0.15	1.66	
5q21.3	117.51	D5S1466	0.9	0	2.03	< 1
5q22.3	123.45	D5S1720	0.86	0.04		
5q23.1	127.93	D5S494	0.92	0.06		
5q23.1	129.83	D5S1505	0.95	0.10	1.52	
5q23.1	130.38	D5S657	0.95	0.02		< 1
5q23.2	130.94	D5S818	0.95	0.02	2.20	1.0
6p24.1	26.71	D6S1006	0.94	0.56	2.37	1.03
6p22.3	29.93	D6S260	0.96	0.61		
6p22.3	32.62	D6S274	0.92	0.92		2.22
	33.26	•	0.87	0.97		
6p22.3	34.23	D6S1959	0.89	0.92	2.41	1.76

Markers flanking maximal MLS are listed. If maximum MLS are obtained between markers, these are included (marked by •, ′). The original genomescan (9) found an MLS of 2.02 between D1S1646 and D1S2667, but did not genotype D1S508. Genetic positions for markers are obtained from Marshfield Clinic, cytogenetic positions from UCSC (<http://genome.ucsc.edu>, March 2006 assembly). Information content (info) is given for all suggestive positions.

Discussion

Many regions have been suggested as candidates to hold genes involved in asthma. No linkage studies have been replicated unambiguously. Our data show suggestive evidence for linkage (MLS 2.92) using a sample of 136 asthmatic sib pairs in a region on chromosome Xp21. The region was also indicated as a candidate region in an earlier genome-wide scan in a different Danish population investigating linkage to asthma (9). Neither of the two studies reached genome significance for chromosome Xp but it is notable that the same region shows evidence for linkage in two independent samples. Weaker evidence for linkage was furthermore observed 10 cM distal to the region just described but did not reach levels of suggestive evidence for linkage. An increased lodscore in this distal region was interestingly also seen in the Danish genome scan (9).

To our knowledge no other group has reported evidence for linkage to asthma or atopy on chromosome Xp. The chromosome has however, only been investigated in a limited number of genome-wide scans.

Chromosome X is, nevertheless, potentially very interesting as gender differences in asthma prevalence and severity are well known (13). Multiple studies show excess asthma in women in adulthood and associations between specific factors related to female reproduction and severity and course of asthma (13). The observed differences between the sexes are likely a product of biological sex differences and sociocultural and environmental differences. As the biological sex differences could partly be

due to genetic risk factors a risk gene on chromosome X seems like an obvious candidate.

Multiple groups have suggested the regions on chromosome 5q and 6p as candidates to harbour genes involved in the development of asthma and two genes from the regions have been identified by positional cloning (5, 14). As in several other reports we were unable to replicate the linkage results in the candidate regions on chromosome 5q15-q23.2 and 6p24.3-p22.3 known from our genome scan in the independent sib pair sample (9). A meta-analysis for linkage to asthma and atopy in the region 5q31-q33, did not find support for the region either (15).

Failure to replicate linkage does not necessarily disprove a hypothesis. Linkage will often involve weak effects, which may turn out to be weaker in a second study. The failure to replicate could be due to etiological heterogeneity or in differences in phenotype definition.

To summarize, our results provide suggestive evidence for linkage to asthma to chromosome Xp21 in a replication study. The fact that the same locus was suggested by two independent studies in the Danish population shows strong evidence for an asthma gene on chromosome Xp21.

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