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Meta-analysis of genome-wide linkage studies of asthma and related traits

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Published: 28 April 2008

Received: 10 December 2007

Respiratory Research 2008, **9**:38 doi:10.1186/1465-9921-9-38

Accepted: 28 April 2008

This article is available from: <http://respiratory-research.com/content/9/1/38>

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Abstract

Background: Asthma and allergy are complex multifactorial disorders, with both genetic and environmental components determining disease expression. The use of molecular genetics holds great promise for the identification of novel drug targets for the treatment of asthma and allergy. Genome-wide linkage studies have identified a number of potential disease susceptibility loci but replication remains inconsistent. The aim of the current study was to complete a meta-analysis of data from genome-wide linkage studies of asthma and related phenotypes and provide inferences about the consistency of results and to identify novel regions for future gene discovery.

Methods: The rank based genome-scan meta-analysis (GSMA) method was used to combine linkage data for asthma and related traits; bronchial hyper-responsiveness (BHR), allergen positive skin prick test (SPT) and total serum Immunoglobulin E (IgE) from nine Caucasian asthma populations.

Results: Significant evidence for susceptibility loci was identified for quantitative traits including: BHR (989 pedigrees, $n = 4,294$) 2p12-q22.1, 6p22.3-p21.1 and 11q24.1-qter, allergen SPT (1,093 pedigrees, $n = 4,746$) 3p22.1-q22.1, 17p12-q24.3 and total IgE (729 pedigrees, $n = 3,224$) 5q11.2-q14.3 and 6pter-p22.3. Analysis of the asthma phenotype (1,267 pedigrees, $n = 5,832$) did not identify any region showing genome-wide significance.

Conclusion: This study represents the first linkage meta-analysis to determine the relative contribution of chromosomal regions to the risk of developing asthma and atopy. Several significant results were obtained for quantitative traits but not for asthma confirming the increased phenotype and genetic heterogeneity in asthma. These analyses support the contribution of regions that contain previously identified asthma susceptibility genes and provide the first evidence for susceptibility loci on 5q11.2-q14.3 and 11q24.1-qter.

Background

Asthma is a disease characterised by recurrent respiratory symptoms, reversible variable airway obstruction, airway inflammation and increased bronchial hyper-responsiveness [1]. Estimates suggest that 100–150 million people worldwide have asthma. Atopy is a predisposition towards the development of immediate hypersensitivity against common environmental antigens. Atopy and asthma are closely related, however they are not interchangeable. Most asthmatic individuals are atopic but atopic individuals may not have asthmatic symptoms. Asthma and atopic disease show strong familial aggregation and heritability estimates vary between 36–79% [2]. A greater understanding of the genetic basis of asthma and atopy holds great promise for the identification of novel therapeutic targets.

Linkage analysis using short tandem repeats or microsatellites to follow the transfer of genetic information between generations has been used to identify chromosomal regions that potentially contain asthma and atopy susceptibility genes. Commonly sub-phenotypes of clinical relevance are used including; elevated total Immunoglobulin E (IgE) levels, atopy defined by positive skin prick test to one or more allergen or elevated specific IgE and bronchial hyper-responsiveness (BHR) [3]. These studies have identified linkage on multiple chromosomal regions *e.g.* 2q22-33, 5q31.1-33, 6p21.3, 11q13, 12q14.3-24.1, 13q14, 14q11.2-13 and 19q13; however replication of linkage findings has been limited [3]. Low statistical power and the potential for type I and type II errors may explain these findings. Combining data has the potential to provide inferences about the consistency of results across studies and to identify regions that contain asthma and atopy susceptibility genes.

The aim of the current study was to complete the first meta-analysis of available genome wide linkage data for asthma and related traits (asthma *per se*, BHR, total IgE, allergen skin prick test response (SPT)) in the Caucasian population using the Genome Scan Meta Analysis (GSMA) method [4]. GSMA is a non parametric, rank based approach and has been used extensively in other disorders *e.g.* schizophrenia [5].

Methods

Systematic Literature Search

To identify published studies for inclusion in the GSMA of asthma and related phenotypes we completed a systematic literature review in September 2006. We used PubMed and the following search string (*Asthma OR BHR OR bronchial hyper responsiveness OR bronchial hyperreactivity OR AHR OR airway hyper responsiveness OR respiratory hypersensitivity OR histamine OR slope OR methacholine OR atopy OR atopic OR dermatitis, atopic OR IgE OR immu-*

noglobulin E OR SPT OR skin prick tests OR skin tests) AND linkage AND genome-scan OR scan OR genome OR genome-wide OR genome-wide OR LOD OR microsatellite). Limits were set on the search including; published in English, human studies, published 1996–2006 and the exclusion of reviews. This initial search identified 516 matches of which 488 were discarded as not containing genome-wide linkage data. A further eight studies were discarded as they were in non-Caucasian populations and we wished to avoid any population stratification issues leaving 20 potential Caucasian studies for inclusion. Genome-wide linkage analyses for asthma related traits in the Hutterite Founder population [6] was not included in the current analyses as limited data was available and the focus of the present study was Caucasian out-bred populations.

Of the 20 manuscripts identified a further nine were removed from the analyses for a combination of the following reasons; the study was superseded by another including the families from the original, LOD score plots in the manuscript were not labelled and/or unreadable, no genome-wide data was presented *e.g.* in the manuscript describing the positional cloning of *ADAM33*, linkage analyses in 460 families for asthma, IgE and BHR phenotypes were performed but has never been published in full [7] or the phenotypes studied did not meet our criteria. All authors were contacted and invited to provide complete datasets.

Phenotype definition and study inclusion/exclusion

There was a large degree of heterogeneity in phenotype definitions and so these were standardised for inclusion. Asthma was defined using doctor diagnosis and/or currently taking asthma medication, however we did include data from the Dutch population which used an algorithm based on asthma symptoms, the presence of BHR, reversibility to β_2 -adrenergic receptor agonist and smoking history to define asthma [8]. Analyses were completed with and without the Dutch families. Total IgE levels were analysed in the genome scans using quantitative data generated by Pharmacia CAP system [9], Pharmacia IgE EIA [10], Phadebas PRIST [11] and ELISA techniques [12,13] which have shown good inter assay correlation [14,15], therefore all studies were included. Positive skin prick response to one or more allergen was used as a marker of atopy and for inclusion in the GSMA. However, allergens used in each study varied; *Dermatophagoides pteronyssinus*, mixed grass pollen [9], *Dermatophagoides pteronyssinus*, *Cladosporium herbarum*, *Alternaria tenuis*, timothy grass, olive, birch, *Parietaria judaica*, ragweed, *Aspergillus*, *Blattella Germanica* [11], mixed grass and tree pollens, mixed weeds, *Dermatophagoides pteronyssinus*, dog, cat, a mixture of guinea pig and rabbit, horse, *Aspergillus fumigatus*, *Alternaria alternata* [10], house dust mite [12], *Dermatophagoides pteronyssinus* and 10 others [13] and

Dermatophagoides pteronyssinus, D. farinae, dog, cat, grass mix, pollen and alternaria [16]. BHR was measured in multiple ways using different provocation stimuli *e.g.* histamine or methacholine providing categorical and/or quantitative analyses. These provocation stimuli have shown a significant correlation ($r = 0.95$) in the responses induced [17], however it is worth noting that this has not been reproduced in all studies. Studies with BHR data were included in the GSMA irrespective of the criteria used in the original manuscript.

Genome Scan Meta Analysis (GSMA)

GSMA was used as it is able to combine linkage data from studies with different marker sets and analysed by different methods including permuted p -values. GSMA was implemented using GSMA software [4,5,18]. Briefly, the genome was divided into 120 bins of approximately 30 cM, for each study the maximum evidence for linkage *e.g.* LOD score or p -value was identified for each bin and these bins were then ranked relative to their evidence for linkage in that study. These ranks were summed across studies and the summed rank (SR) forms the basis of the test statistic [4]. An ordered rank (OR) statistic was also generated which gives a genome wide interpretation of significance by comparing the n -th highest summed rank with the distribution of the n -th highest summed ranks obtained through simulation [5]. We completed an unweighted and weighted analyses using information content ($\sqrt{(\text{no. pedigrees} \times \text{no. markers})}$) as a weighting factor.

Statistical Significance

Simulation studies have shown that any bin with a $p(\text{SR}) < 0.05$ and a $p(\text{OR}) < 0.05$ has a high probability of containing a true susceptibility gene [5]. Applying Bonferroni correction a $p < 0.000417$ provides evidence for genome wide significance for linkage and a $p < 0.0083$ provides suggestive evidence for linkage [5].

Results

Data included

The selection criteria and data requests provided eleven studies of nine Caucasian asthma populations for our analyses (Table 1) including data from 1,267 pedigrees ($n = 5,832$) for asthma (80.2% of pedigrees available in the public domain or following request, missing 249 [19] and 65 pedigrees [20]), 989 pedigrees ($n = 4,294$) for BHR (79.9% of available, missing 249 pedigrees [19]), 1,093 pedigrees ($n = 4,746$) for SPT (81.5% of available, missing 249 pedigrees [19]) and 729 pedigrees ($n = 3,224$) for total IgE (65.9% of available, missing 249 [19], and 129 pedigrees [21]).

Asthma

The weighted asthma analyses did not identify any chromosomal region with a $p(\text{SR})$ and $p(\text{OR}) < 0.05$ (Figure 1 and Table 2). No bin $p(\text{SR})$ met genome wide significance ($p < 0.000417$) or suggestive evidence for linkage ($p < 0.0083$) in these analyses however three regions demonstrated a $p(\text{SR}) < 0.05$; 6p22.3-p21.1, 10p14-q11.21 and 12q24.31-qter (Table 2). Eight regions met suggestive linkage criteria in the ordered rank analyses; 1p31.1-p13.3, 2p12-q22.1, 4p14-q13.3, 7q34-qter, 12pter-p12.1, 12p12.1-p11.21, 14q32.12-qter, 17pter-p12 and 20pter-p12.3 (Table 2). Analyses of the asthma phenotype using unweighted GSMA generated similar findings to the weighted analyses (Figure 2). To confirm that the inclusion of the Dutch linkage data for the asthma phenotype (defined by algorithm) had not confounded the analyses we completed GSMA without these data focusing on doctor diagnosed asthma only (1,067 pedigrees). Again, no chromosomal region with a $p(\text{SR})$ and $p(\text{OR}) < 0.05$ was identified (data not shown).

Bronchial Hyper-responsiveness

The weighted BHR analyses strongly suggested that 6p22.3-p21.1 contains BHR susceptibility gene(s) as a $p(\text{SR})$ and $p(\text{OR}) < 0.05$ was observed ($p = 0.007$, $p = 0.049$ respectively (Figure 1 and Table 3)). Two other regions showed suggestive evidence ($p < 0.0083$) for linkage to the BHR phenotype; 2p12-q22.1 ($p(\text{SR}) = 0.006$) and 11q24.1-qter ($p(\text{SR}) = 0.005$). In the unweighted analyses three regions showed evidence for linkage ($p(\text{SR})$ and $p(\text{OR}) \leq 0.05$), *i.e.* 2q22.1-q23.3, 7q12.11-q31.1 and 5q23.2-q34 (Figure 2).

Positive allergen skin prick test

Weighted analyses of the SPT phenotype identified two regions that had a $p(\text{SR})$ and $p(\text{OR}) \leq 0.05$ strongly suggesting these regions are susceptibility loci (Figure 1 and Table 4). These regions are 17p12-q24.3 (two adjacent bins, 17p12-q21.33 $p(\text{SR}) = 0.00043$, $p(\text{OR}) = 0.050$ and 17q21.33-q24.3 $p(\text{SR}) = 0.047$, $p(\text{OR}) = 0.038$) and region 3p22.1-q22.1 (three adjacent bins, 3p22.1-p14.1 $p(\text{SR}) = 0.045$, $p(\text{OR}) = 0.063$, 3p14.1-q12.3 $p(\text{SR}) = 0.003$, $p(\text{OR}) = 0.0045$ and 3q12.3-q22.1 $p(\text{SR}) = 0.00084$, $p(\text{OR}) = 0.00406$). The analyses of the unweighted SPT datasets identified chromosomes 3 and 17 as containing the major determinants (Figure 2).

Total Immunoglobulin E

Weighted analyses of the IgE phenotype strongly suggested 5q11.2-q14.3 ($p(\text{SR}) = 0.031$, $p(\text{OR}) = 0.060$) and 6pter-p22.3 ($p(\text{SR}) = 0.033$, $p(\text{OR}) = 0.026$) contain genes that influence IgE levels (Figure 1 and Table 5). The region adjacent to 6pter-p22.3, *i.e.* 6p22.3-p21.1 has a $p(\text{SR}) = 0.00999$ approaching suggestive linkage providing further evidence for this region. Analyses of the IgE

Table 1: Characteristics of genome-wide linkage studies included in the GSMA

| COHORT (REF.) | PHENOTYPES ^(a) | NUMBER OF ^(b) | | | MKR MAP (SP.) ^(c) | STUDY TYPE ^(d) | ANALYSIS PROGRAM | INCLUDED IN GSMA |
|--------------------|--|--------------------------|------|---------|------------------------------|---------------------------|----------------------------|--|
| | | PED. (AFF/IND.) | MKS. | WF. | | | | |
| Australian [9] | B: Slope (-, 0–12 μmol meth.) I: Quantitative S: 2 allergens | 80 (25/364) | 253 | 142.267 | - (10 cM) | Nonpar. two. | - | B: 23 p-values < 0.05 I: 21 p-values < 0.05 S: 19 p-values < 0.05 |
| CSGA [33] | A: Quest./Doc diag | 79 (200/316) | 360 | 168.642 | Marshfield (10 cM) | Nonpar. multi. | Modified GENEHUNTER | A: LOD scores from graphs |
| German [34] | B: PD ₂₀ (Neb, < 8 mg/ml meth.) | 97 (200/415) | 333 | 179.725 | Modified Genethon (10.7 cM) | Nonpar. multi. | GENEHUNTER, MAP-MAKER/SIBS | B: Complete dataset |
| French [11] | A: Quest./Meds. B: Slope (-, meth.) I: Quantitative S: 11 allergens | 46 (102/210) | 254 | 108.093 | - (13 cM) | Nonpar. multi. | GENEHUNTER, SIBPAIR | A: 11 p-values B: 8 p-values I: 8 p-values S: 19 p-values |
| Icelandic [35] | A: Doc diag./Meds. | 175 (596/1134) | 976* | 413.280 | Decode (4 cM) | Nonpar. multi. | ALLEGRO | A: LOD scores from graphs |
| Dutch [10, 36, 37] | A: Algorithm B: PD ₂₀ (-, < 32 mg/ml hist.) I: Quantitative S: 16 allergens | 200 (-/1159) | 366 | 270.555 | Marshfield Weber v8 (10 cM) | Nonpar. | SOLAR, GENEHUNTER | A: Complete dataset B: Complete dataset I: Complete dataset S: Complete dataset |
| German [12] | A: Doc diag. I: Quantitative/Catagorical S: HDM+ | 201 (506/867) | 364 | 270.489 | Modified Genethon (10 cM) | Nonpar. multi. | MERLIN | A: Complete dataset I: Complete quantitative dataset S: Complete dataset |
| Australian [13] | A: Quest./Meds. B: PD ₂₀ (-, < 7.8 μmol hist) I: Quantitative S: 11 allergens. | 202 (169/591) | 624 | 355.032 | - (7.1 cM) | Nonpar. multi. | MERLIN, SOLAR | A: Complete dataset B: Complete dataset I: Complete dataset S: Complete dataset |
| GAIN [16] | A: Doc diag. B: PD ₂₀ (-, < 8 mg/ml meth.) S: 7 allergens | 364 (1014/1555) | 396* | 379.663 | Decode (-) | Nonpar. multi. | MERLIN | A: LOD scores from graphs B: LOD scores from graphs S: LOD scores from graphs |

(a) A = asthma; B = bronchial hyper-responsiveness (Neb = nebuliser, Dos = dosimeter); I = total serum IgE; S = skin prick test response; Quest = questionnaire; Doc diag = doctor diagnosis; Meds = asthma medication; Meth = methacholine; Hist = histamine; HDM = house dust mite; PD₂₀ = provocation dose resulting in a 20% fall in FEV₁. (b) Ped = total genotyped pedigrees; AFF = total genotyped asthmatic cases, Ind = total genotyped individuals; Mks = total autosomal microsatellite markers; Wt = weighting factor. (c) Sp = average marker spacing. (d) Nonpar = nonparametric; Two = two point; Multi = multipoint. (-) = not provided, *number of autosomal markers not given, total number used for weighting.

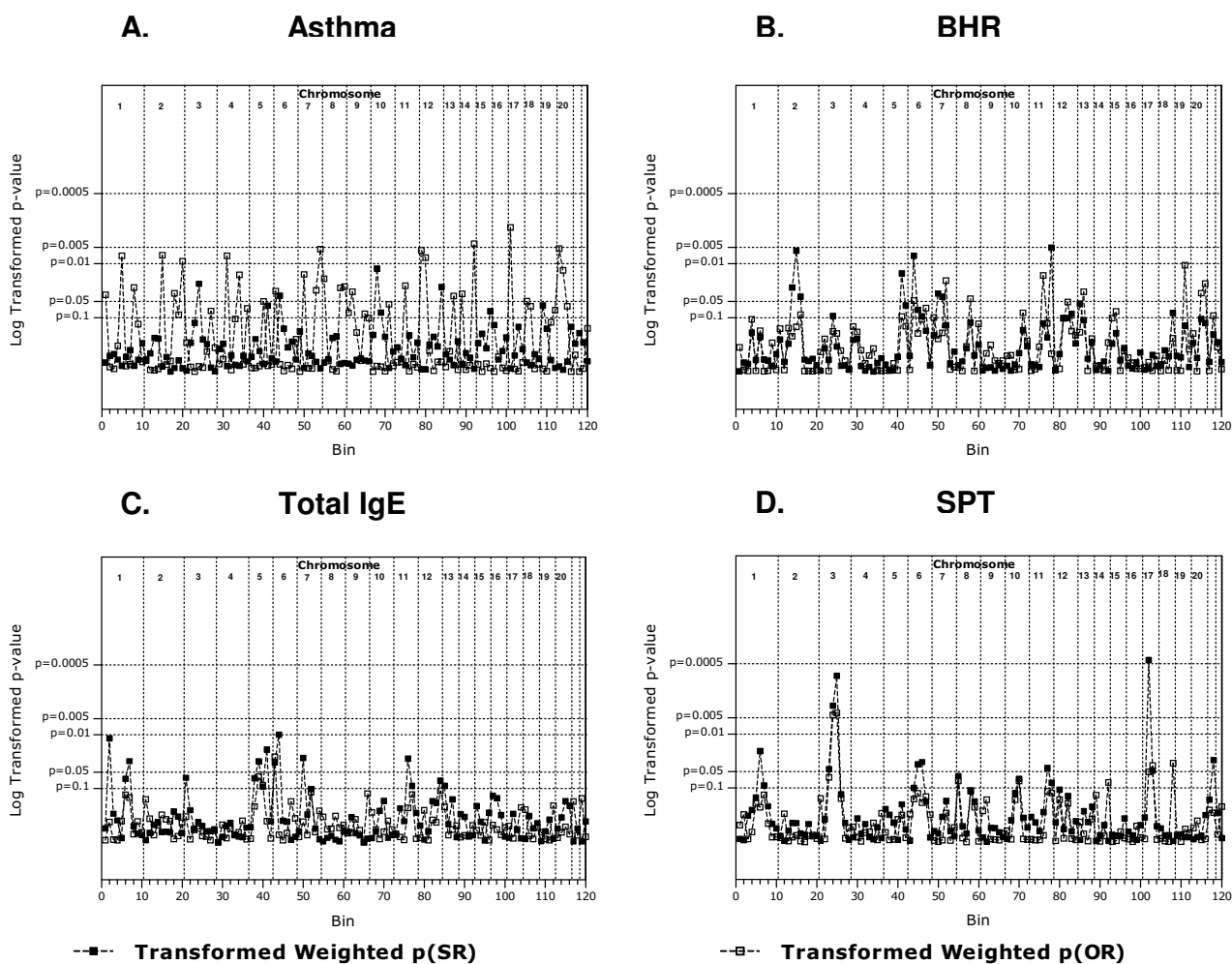


Figure 1
p(SR) & p(OR) in weighted GSMA. A. asthma. B. bronchial hyper-responsiveness. C. total serum IgE. D. skin prick test response. A p(SR) of $p < 0.000417$ = significant linkage, $p < 0.0083$ = suggestive linkage. p(SR) & p(OR) data were transformed using $f(x) = 0.05/x$ and plotted on a \log_{10} scale to improve clarity.

phenotype using the unweighted GSMA showed similar overall findings (Figure 2).

Multiple phenotype analyses

In addition to the primary phenotype analyses we investigated overlapping chromosomal regions containing genetic determinants of asthma and asthma related traits consistent with gene(s) having pleiotrophic effects in asthma and allergy (Table 6). Interestingly, region 6p22.3-p21.1 which contains the HLA region showed a p(SR) < 0.05 in the asthma, BHR and IgE analyses potentially as expected due to the role of HLA restriction in many immunological mechanisms. Several other regions also showed overlapping concordance, in particular regions; 3p14.1-q12.3 (asthma, SPT), 5q23.2-q34 (asthma, BHR, IgE) and 7p21.1-14.1 (asthma, BHR, IgE).

Discussion

This study represents the first meta-analysis of asthma and related trait linkage data using the majority of the data available for the Caucasian asthma cohorts in the public domain. This analysis combines data from 10 years of asthma and atopy genetics and is extremely timely providing a definitive analysis of available linkage data to complement the highly anticipated whole genome association findings. Analysis of asthma and atopy quantitative traits identified significant evidence for relatively few chromosomal regions as containing susceptibility gene(s) using the most stringent genome-wide criteria *i.e.* BHR (6p22.3-p21.1), total IgE (5q11.2-q14.3 and 6pter-p22.3) and positive allergen skin prick test (3p22.1-q22.1, 17p12-q24.3). Significantly no chromosomal region met stringent genome-wide criteria in the asthma phenotype anal-

Table 2: Weighted GSMA for Asthma (1,267 pedigrees, n = 5,832)

| BIN ^(a) | GENETIC LOCUS ^(b) | DISTANCE (cM) ^(c) | PHYSICAL POSITION (KB) ^(d) | SUMMED RANK (SR) | PROBABILITY | |
|--------------------|------------------------------|------------------------------|---------------------------------------|------------------|-------------|----------|
| | | | | | p(SR) | p(OR) |
| 1 | 1pter-p36.23 | 0.00-20.61 | pter-9332 | 376.576 | 0.68121 | 0.03775 |
| 5 | 1p31.1-p13.3 | 113.69-142.24 | 82867-110103 | 346.834 | 0.78259 | 0.00718* |
| 8 | 1q31.1-q32 | 201.58-231.11 | 185232-210150 | 347.872 | 0.77937 | 0.02791 |
| 15 | 2p12-q22.1 | 101.56-128.41 | 79617-119705 | 341.174 | 0.79974 | 0.00694* |
| 18 | 2q31.1-q34 | 177.53-206.74 | 172805-212021 | 317.985 | 0.86170 | 0.03548 |
| 20 | 2q35-qter | 233.62-269.07 | 230618-qter | 316.169 | 0.86603 | 0.00908 |
| 24 | 3p14.1-q12.3 | 88.60-117.76 | 64182-103187 | 612.921 | 0.02348 | 0.80059 |
| 31 | 4p14-q13.3 | 51.60-78.97 | 38279-72275 | 343.939 | 0.79152 | 0.00714* |
| 34 | 4q28.3-q32.1 | 134.74-159.30 | 137492-160828 | 346.992 | 0.78212 | 0.01609 |
| 43 | 6pter-p22.3 | 0.00-32.62 | pter-16854 | 383.747 | 0.65433 | 0.03220 |
| 44 | 6p22.3-p21.1 | 32.62-65.14 | 16854-43207 | 592.740 | 0.03905 | 0.72593 |
| 50 | 7p21.1-p14.1 | 29.28-59.93 | 19430-40230 | 317.779 | 0.86218 | 0.01583 |
| 53 | 7q31.1-q34 | 122.48-148.11 | 75216-138638 | 381.650 | 0.66230 | 0.03107 |
| 54 | 7q34-qter | 148.11-181.97 | 138638-qter | 313.741 | 0.87152 | 0.00536* |
| 55 | 8pter-p22 | 0.00-27.40 | pter-13111 | 376.368 | 0.68196 | 0.01899 |
| 59 | 8q22-q24.21 | 110.2-137.92 | 99237-127416 | 365.804 | 0.71999 | 0.02820 |
| 60 | 8q24.21-qter | 137.92-167.90 | 127416-qter | 373.141 | 0.69378 | 0.02636 |
| 62 | 9p22.3-p21.1 | 27.32-53.60 | 14264-29850 | 349.926 | 0.77291 | 0.03321 |
| 68 | 10p14-q11.21 | 29.15-62.23 | 10591-36230 | 635.659 | 0.01229 | 0.79026 |
| 75 | 11p12-q13.3 | 47.06-72.82 | 36450-70234 | 353.473 | 0.76155 | 0.02578 |
| 79 | 12pter-p12.1 | 0.00-24.45 | pter-11686 | 299.278 | 0.90180 | 0.00570* |
| 80 | 12p12.1-p11.21 | 24.45-53.28 | 11686-32879 | 292.228 | 0.91466 | 0.00766* |
| 84 | 12q24.31-qter | 139.61-170.60 | 120806-qter | 607.388 | 0.02714 | 0.65821 |
| 87 | 13q22.2-q33.1 | 58.54-85.41 | 74875-102346 | 354.469 | 0.75831 | 0.04007 |
| 89 | 14pter-q13.1 | 0.00-40.11 | pter-33529 | 362.366 | 0.73182 | 0.03622 |
| 92 | 14q32.12-qter | 105.00-138.18 | 90647-qter | 305.539 | 0.88932 | 0.00423* |
| 101 | 17pter-p12 | 0.00-25.14 | pter-11325 | 312.768 | 0.87374 | 0.00213* |
| 105 | 18pter-p11 | 0.00-24.08 | pter-7462 | 377.842 | 0.67650 | 0.04965 |
| 113 | 20pter-p12.3 | 0.00-21.15 | pter-7608 | 339.111 | 0.80587 | 0.00524* |
| 114 | 20p12.3-p11 | 21.15-47.52 | 7608-21259 | 282.935 | 0.92982 | 0.01324 |

Chromosomal regions with a p(SR) or p(OR) < 0.05 are shown. ^aBin number from GSMA (1-120 inclusive). ^bCytogenetic band (Taken from April 2002 Genome Browser, UCSC). ^cGenetic distance in Marshfield cM (not cumulative). ^dPhysical position in kilobase pairs (Taken from December 2006 UniSTS, NCBI/Genome Browser, UCSC). *p < 0.000417 = Genome wide significance for linkage, p < 0.0083 = Suggestive for linkage [5].

Table 4: Weighted GSMA for skin prick test response (1,093 pedigrees, n = 4,746)

| BIN ^(a) | GENETIC LOCUS ^(b) | DIST. (cM) ^(c) | PHYS POS. (KB) ^(d) | SUMMED RANK (SR) | PROBABILITY | |
|--------------------|------------------------------|---------------------------|-------------------------------|------------------|-----------------|----------------|
| | | | | | p(SR) | p(OR) |
| 6 | 1p13.3-q23.3 | 142.24-170.84 | 110103-159125 | 541.271 | 0.02081 | 0.22906 |
| 23 | 3p22.1-p14.1 | 63.12-88.60 | 38845-64182 | 513.869 | 0.04488 | 0.06344 |
| 24 | 3p14.1-q12.3 | 88.60-117.76 | 64182-103187 | 591.392 | 0.00298* | 0.00450 |
| 25 | 3q12.3-q22.1 | 117.76-146.60 | 103187-134474 | 614.373 | 0.00084* | 0.00406 |
| 45 | 6p21.1-q15 | 65.14-99.01 | 43207-90985 | 521.717 | 0.03654 | 0.12598 |
| 46 | 6q15-q23.2 | 99.01-131.07 | 90985-132584 | 525.276 | 0.03318 | 0.18839 |
| 77 | 11q22.3-q24.1 | 98.98-123.00 | 103732-122977 | 515.746 | 0.04276 | 0.11460 |
| 102 | 17p12-q21.33 | 25.14-63.62 | 11325-39726 | 624.777 | 0.00043* | 0.05044 |
| 103 | 17q21.33-q24.3 | 63.62-93.98 | 39726-66600 | 511.187 | 0.04799 | 0.03824 |
| 108 | 18q22.1-qter | 96.48-126.00 | 60025-qter | 209.839 | 0.96132 | 0.03480 |
| 118 | 21q21.3-qter | 25.26-57.77 | 27903-qter | 528.536 | 0.03029 | 0.28738 |

Chromosomal regions with a p(SR) or p(OR) < 0.05 are shown. ^aBin number from GSMA (1-120 inclusive). ^bCytogenetic band (Taken from April 2002 Genome Browser, UCSC). ^cGenetic distance in Marshfield cM (not cumulative). ^dPhysical position in kilobase pairs (Taken from December 2006 UniSTS, NCBI/Genome Browser, UCSC). *p < 0.000417 = Genome wide significance for linkage, p < 0.0083 = Suggestive for linkage [5].

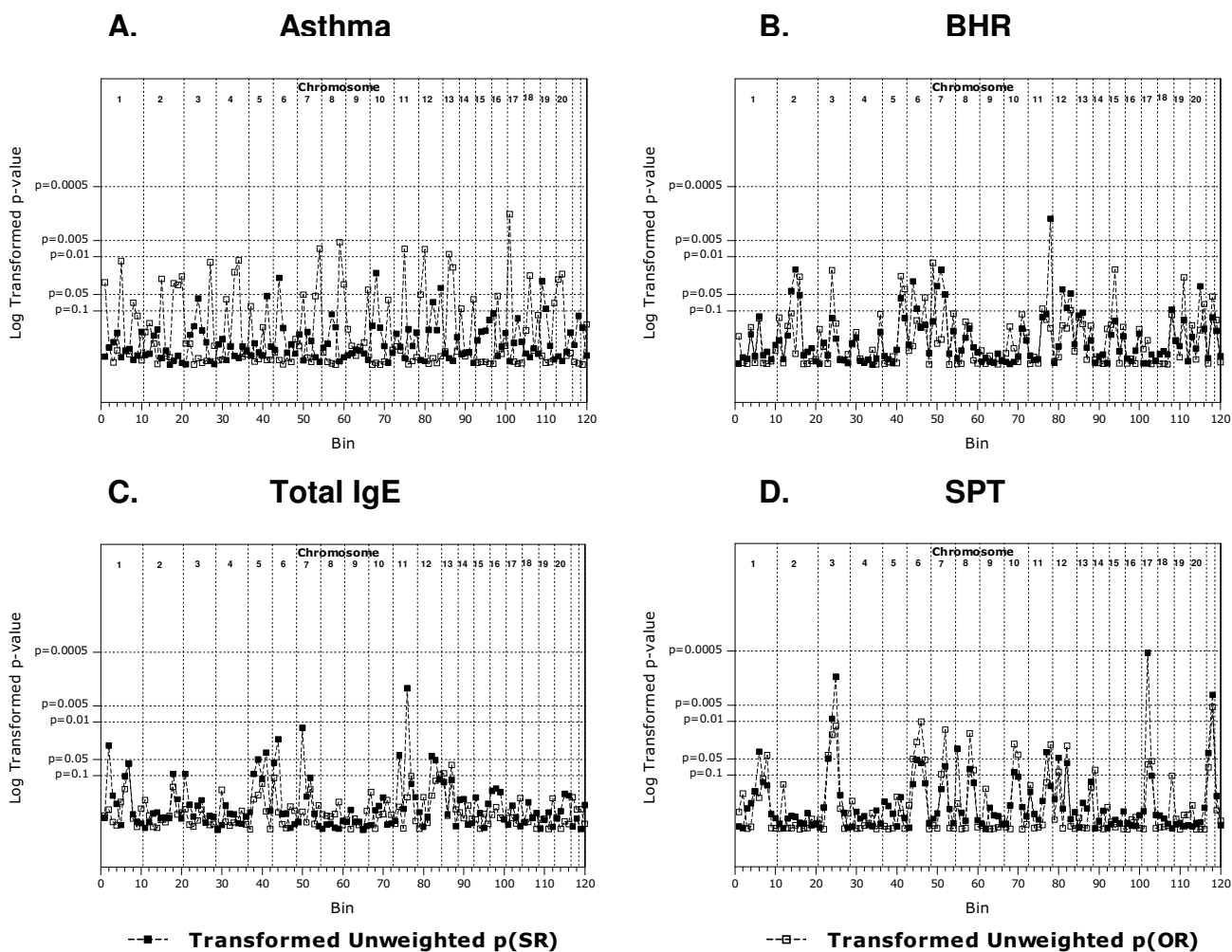


Figure 2
p(SR) & p(OR) in unweighted GSMA. A. asthma. B. bronchial hyper-responsiveness. C. total serum IgE. D. skin prick test response. A p(SR) of $p < 0.000417$ = significant linkage, $p < 0.0083$ = suggestive linkage. p(SR) & p(OR) data were transformed using $f(x) = 0.05/x$ and plotted on a \log_{10} scale to improve clarity.

yses. This study did provide supporting evidence for regions containing previously identified asthma susceptibility genes.

Linkage analyses has proven to be highly successful in single gene disorders e.g. cystic fibrosis but has been problematic in asthma and atopy mainly due to the complex genetic basis of these phenotypes and the use of inadequate samples sizes leading to both type I and type II errors. In the current study we aimed to combine all available linkage data for asthma and related trait phenotypes (BHR, total IgE, positive allergen skin prick test) and provide inferences about the consistency of results across studies, ultimately providing a focus for future gene discovery. The analyses of the quantitative traits provided the most significant findings and may be consistent with the

observation that using objective markers of disease adds to the homogeneity of the data and may improve results. In addition the number of genes regulating these phenotypes may be smaller than "asthma" and power to find these may be increased.

This study strongly suggested that regions 17p12-q21.33 and 3p14.1q22.1 contain gene(s) that influence allergen skin prick responses and by inference atopy. Both of these regions are large spanning 28.5 and 70.3 Mbp respectively. The 3p21 region has been identified as containing genetic determinants of specific allergen responses in the Hutterite founder asthma cohort [22] and has been identified as an atopic dermatitis locus (3p24-22) [23]. Linkage to chromosome 17 and specific allergen responses has been described in the Hutterite population however these

Table 3: Weighted GSMA for bronchial hyper-responsiveness (989 pedigrees, n = 4,294)

| BIN ^(a) | GENETIC LOCUS ^(b) | DISTANCE (cM) ^(c) | PHYSICAL POSITION (KB) ^(d) | SUMMED RANK (SR) | PROBABILITY | |
|--------------------|------------------------------|------------------------------|---------------------------------------|------------------|-----------------|----------------|
| | | | | | p(SR) | p(OR) |
| 14 | 2p16.2-p12 | 76.34-101.56 | 54063-79617 | 532.855 | 0.02751 | 0.22094 |
| 15 | 2p12-q22.1 | 101.56-128.41 | 79617-119705 | 576.585 | 0.00574* | 0.14743 |
| 16 | 2q22.1-q23.3 | 128.41-154.48 | 119705-151529 | 518.593 | 0.04085 | 0.08859 |
| 41 | 5q23.2-q34 | 131.48-164.19 | 123774-162087 | 551.701 | 0.01511 | 0.09586 |
| 44 | 6p22.3-p21.1 | 32.62-65.14 | 16854-43207 | 571.381 | 0.00716* | 0.04861 |
| 50 | 7p21.1-p14.1 | 29.28-59.93 | 19430-40230 | 523.367 | 0.03597 | 0.24658 |
| 51 | 7p14.1-q21.11 | 59.93-91.67 | 40230-81002 | 518.746 | 0.04070 | 0.19114 |
| 52 | 7q21.11-q31.1 | 91.67-122.48 | 81002-109766 | 461.380 | 0.13926 | 0.02065 |
| 58 | 8q13.1-q22 | 82.84-110.20 | 67744-99237 | 468.188 | 0.12312 | 0.04443 |
| 76 | 11q13.3-q22.3 | 72.82-98.98 | 70185-103732 | 465.589 | 0.12913 | 0.01652 |
| 78 | 11q24.1-qter | 123.00-147.77 | 122977-qter | 579.520 | 0.00505* | 0.46485 |
| 86 | 13q13.2-q22.2 | 26.87-58.54 | 33636-74875 | 472.999 | 0.11255 | 0.03312 |
| 111 | 19q12-q13.33 | 52.59-75.41 | 35843-54589 | 460.759 | 0.14078 | 0.01070 |
| 115 | 20p11-q13.13 | 47.52-72.27 | 21259-46747 | 476.308 | 0.10563 | 0.03503 |
| 116 | 20q13.13-qter | 72.27-101.22 | 46747-qter | 467.593 | 0.12448 | 0.02343 |

Chromosomal regions with a p(SR) or p(OR) < 0.05 are shown. ^aBin number from GSMA (1-120 inclusive). ^bCytogenetic band (Taken from April 2002 Genome Browser, UCSC). ^cGenetic distance in Marshfield cM (not cumulative). ^dPhysical position in kilobase pairs (Taken from December 2006 UniSTS, NCBI/Genome Browser, UCSC). *p < 0.000417 = Genome wide significance for linkage, p < 0.0083 = Suggestive for linkage [5].

linkages map to 17q25 in asthma [22]. Linkage to atopic dermatitis on 17q23.1 has been reported [23]. Also of significance is the fact that the chromosome 17 locus (17p12-q21.33) identified in the current analyses contains the recently identified *ORMDL3* gene [24]. Using whole genome association variants in the *ORMDL3* gene were shown to be associated with childhood onset asthma [24]. Overall our data suggest that the major genes influencing allergen skin prick responses are found on chromosomes 3 and 17.

In the total IgE analyses there was strong evidence for the presence of genes(s) regulating IgE production in the 5q11.2-q14.3 and 6pter-p21.1 regions. This region on chromosome 6 contains the Human Leukocyte Antigen

(HLA) locus and so may be predicted to contain determinants of immunological processes. The finding that 5q11.2-q14.3 may contain gene(s) that influence IgE production is novel and warrants further investigation. The IgE analyses also confirmed the potential contribution of genes within the 5q23.2-q34 and 11q13.3-q22.3 regions that have previously been suggested [25]. Interestingly, of the four positionally cloned genes identified using IgE as a key phenotype only the region encompassing the *GPR4* gene showed limited (non significant) linkage (7p21.1-p14.1, p(SR) = 0.027).

In the BHR analyses the 6p22.3-p21.1 region was identified as containing susceptibility gene(s). This region contains the HLA locus and the HLA-G gene within this

Table 5: Weighted GSMA for total serum IgE (729 pedigrees, n = 3,224)

| BIN ^(a) | GENETIC LOCUS ^(b) | DIST. (cM) ^(c) | PHYS POS. (KB) ^(d) | SUMMED RANK (SR) | PROBABILITY | |
|--------------------|------------------------------|---------------------------|-------------------------------|------------------|----------------|----------------|
| | | | | | p(SR) | p(OR) |
| 2 | 1p36.23-p35.3 | 20.61-54.30 | 9332 - 24723 | 476.251 | 0.01171 | 0.41867 |
| 7 | 1q23.3-q31.1 | 170.84-201.58 | 159125-185232 | 450.659 | 0.03099 | 0.14664 |
| 39 | 5q11.2-q14.3 | 64.14-97.82 | 55758-88765 | 450.303 | 0.03127 | 0.06031 |
| 41 | 5q23.2-q34 | 131.48-164.19 | 123774-162087 | 464.611 | 0.01890 | 0.40276 |
| 43 | 6pter-p22.3 | 0.00-32.62 | pter-16854 | 448.903 | 0.03276 | 0.02583 |
| 44 | 6p22.3-p21.1 | 32.62-65.14 | 16854-43207 | 479.723 | 0.00999 | 0.71984 |
| 50 | 7p21.1-p14.1 | 29.28-59.93 | 19430-40230 | 454.706 | 0.02708 | 0.41494 |
| 76 | 11q13.3-q22.3 | 72.82-98.98 | 70185-103732 | 453.763 | 0.02792 | 0.22926 |

Chromosomal regions with a p(SR) or p(OR) < 0.05 are shown. ^aBin number from GSMA (1-120 inclusive). ^bCytogenetic band (Taken from April 2002 Genome Browser, UCSC). ^cGenetic distance in Marshfield cM (not cumulative). ^dPhysical position in kilobase pairs (Taken from December 2006 UniSTS, NCBI/Genome Browser, UCSC). *p < 0.000417 = Genome wide significance for linkage, p < 0.0083 = Suggestive for linkage [5].

Table 6: Overlapping chromosomal regions in weighted GSMA for asthma, and the three intermediate phenotypes

| BIN ^(a) | GENETIC LOCUS ^(b) | DIST. (cM) ^(c) | PHYS POS. (KBp) ^(d) | ASTHMA | | BHR | | TOTAL IGE | | SPT RESPONSE | |
|--------------------|------------------------------|---------------------------|--------------------------------|---------|----------|----------|---------|-----------|---------|--------------|----------|
| | | | | p(SR) | p(OR) | p(SR) | p(OR) | p(SR) | p(OR) | p(SR) | p(OR) |
| 6 | 1p13.3-q23.3 | 142.24-170.84 | 110103-159125 | - | - | - | - | 0.06630 | 0.13155 | 0.02081 | 0.22906 |
| 7 | 1q23.3-q31.1 | 170.84-201.58 | 159125-185232 | - | - | - | - | 0.03099 | 0.14664 | 0.08924 | 0.13402 |
| 15 | 2p12-q22.1 | 101.56-128.41 | 79617-119705 | 0.79974 | 0.00694 | 0.00574* | 0.14743 | - | - | - | - |
| 24 | 3p14.1-q12.3 | 88.60-117.76 | 64182-103187 | 0.02348 | 0.80059 | 0.09312 | 0.17837 | - | - | 0.00298 | 0.00450* |
| 40 | 5q5q14.3-q23.2 | 97.82-131.48 | 88765-123774 | 0.71879 | 0.05004 | - | - | 0.09195 | 0.08596 | - | - |
| 41 | 5q23.2-q34 | 131.48-164.19 | 123774-162087 | 0.06027 | 0.77886 | 0.01511 | 0.09586 | 0.01890 | 0.40276 | - | - |
| 43 | 6pter-p22.3 | 0.00-32.62 | pter-16854 | 0.65433 | 0.03220 | - | - | 0.03276 | 0.02583 | - | - |
| 44 | 6p22.3-p21.1 | 32.62-65.14 | 16854-43207 | 0.03905 | 0.72593 | 0.00716* | 0.04861 | 0.00999 | 0.71984 | 0.09934 | 0.16043 |
| 45 | 6p21.1-q15 | 65.14-99.01 | 43207-90985 | - | - | 0.07128 | 0.19832 | - | - | 0.03654 | 0.12598 |
| 46 | 6q15-q23.2 | 99.01-131.07 | 90985-132584 | - | - | 0.09445 | 0.10925 | - | - | 0.03318 | 0.18839 |
| 50 | 7p21.1-p14.1 | 29.28-59.93 | 19430-40230 | 0.86218 | 0.01583 | 0.03597 | 0.24658 | 0.02708 | 0.41494 | - | - |
| 55 | 8pter-p22 | 0.00-27.40 | pter-13111 | 0.68196 | 0.01899 | - | - | - | - | 0.06027 | 0.07341 |
| 71 | 10q23.33-q26.13 | 117.42-142.78 | 95985-123274 | 0.85805 | 0.05664 | 0.16985 | 0.08106 | - | - | - | - |
| 76 | 11q13.3-q22.3 | 72.82-98.98 | 70185-103732 | - | - | 0.12913 | 0.01652 | 0.02792 | 0.22926 | - | - |
| 77 | 11q22.3-q24.1 | 98.98-123.00 | 103732-122977 | - | - | - | - | 0.08890 | 0.12887 | 0.04276 | 0.11460 |
| 78 | 11q24.1-qter | 123.00-147.77 | 122977-qter | - | - | 0.00505* | 0.46485 | - | - | 0.08072 | 0.12542 |
| 84 | 12q24.31-qter | 139.61-170.60 | 120806-qter | 0.02714 | 0.65821 | - | - | 0.07175 | 0.10909 | - | - |
| 85 | 13pter-q13.2 | 0.00-26.87 | pter-33636 | - | - | 0.05521 | 0.18686 | 0.08835 | 0.22042 | - | - |
| 92 | 14q32.12-qter | 105.00-138.18 | 90647-qter | 0.88932 | 0.00423* | - | - | - | - | 0.95708 | 0.07887 |
| 108 | 18q22.1-qter | 96.48-126.00 | 60025-qter | - | - | 0.08190 | 0.24216 | - | - | 0.96132 | 0.03480 |
| 115 | 20p11-q13.13 | 47.52-72.27 | 21259-46747 | 0.65424 | 0.06184 | 0.10563 | 0.03503 | - | - | - | - |
| 118 | 21q21.3-qter | 25.26-57.77 | 27903-qter | - | - | 0.20402 | 0.09140 | - | - | 0.03029 | 0.28738 |

Chromosomal regions with p(SR) or p(OR) < 0.1 in two or more weighted GSMA are shown. ^aBin number from GSMA (1-120 inclusive).

^bCytogenetic band (Taken from April 2002 Genome Browser, UCSC). ^cGenetic distance in Marshfield cM (not cumulative). ^dPhysical position in kilobase pairs (Taken from December 2006 UniSTS, NCBI/Genome Browser, UCSC). *p < 0.000417 = Genome wide significance for linkage, p < 0.0083 = Suggestive for linkage.

region has previously been identified as a potential asthma and BHR susceptibility gene using four cohorts (including the Dutch cohort used in the current analyses) [26]. Our data confirms this region as a BHR locus and less significantly a potential asthma locus (p(SR) = 0.039). Interestingly, four of the six populations used for the BHR analyses ranked the chromosome 2p locus identified in the top 33% of bins including the Genetics of Asthma International Network (GAIN) study (data not shown). Further mapping of the 2p locus in the GAIN population using single nucleotide polymorphisms (SNP) spanning the region refined the linkage peak to ~70 cM with the greatest evidence being for the BHR phenotype (LOD score 4.58) [16]. Region 2p12-q22.1 contains the *DPP10* gene that has previously been identified as an asthma and total IgE susceptibility gene [27] and the *IL1RN* gene identified using asthma as the primary phenotype [28]. The identification of 11q24.1-qter as a potential BHR susceptibility locus appears to be novel and therefore these data may provide a platform for novel BHR susceptibility gene identification. The BHR analyses also confirmed the potentially modest contribution of other loci in determining the BHR phenotype including *e.g.* 5q23.2-q34 and 19q12-q13.33. In the analyses of the

BHR phenotype significant linkage was not driven by studies using a specific agonist *i.e.* studies using both methacholine and histamine provocation contributed to the signal at a specific locus (data not shown). These data suggest responsiveness to these agents share a common genetic basis and provide support for combining studies in the meta-analyses using these different provocation stimuli.

Significantly, using asthma as a phenotype we did not identify any chromosomal region as showing genome-wide significance in our analyses. In most studies, asthma was defined as a doctor's diagnosis. In the Dutch study, families were ascertained through a proband with a doctor's diagnosis of asthma. In the offspring of these asthma patients, an algorithm was used since a doctor's diagnosis *per se* underestimated the prevalence of asthma in the offspring [8]. To confirm that the inclusion of the Dutch linkage data for the asthma phenotype had not confounded the analyses we completed GSMA without these data and again no chromosomal region with a p(SR) and p(OR) < 0.05 was identified (data not shown). These data may reflect the true locus heterogeneity in asthma or reflect differences in phenotype definition when compar-

ing asthma based on doctors' diagnosis over different cohorts. In addition the use of a binary trait *i.e.* affection will have the lowest intrinsic power compared to continual data *e.g.* IgE levels.

Interestingly, the recently published whole genome association study using 994 asthmatic children and 1,243 non asthmatic children identified only 16 SNPs (eight on chromosome 17) from a total of 317,447 SNPs tested showing a significant association with asthma *per se* (5% false discovery threshold, stratification corrected) [24]. This study complements our analysis using data from 1,257 families and taken together suggests that the use of asthma as a phenotype may be confounded due to locus heterogeneity in asthma and/or issues concerning phenotype definition/heterogeneity when combining cohorts. It is important to note that the family based studies included in this meta-analysis address the genetic basis of asthma defined in children *i.e.* the mean age of siblings in most studies is < 16 years.

Several regions showed suggestive evidence for linkage to the asthma phenotype mainly based on the p(OR) statistic, however caution should be taken interpreting p(OR) in isolation, especially in the presence of incomplete data sets [5]. Further evidence for the chromosome 12 and 20 loci comes from the finding that adjacent bins have a p(OR) < 0.05 suggesting the linkage is spanning the bin interval. 4/6 regions containing the positionally cloned asthma susceptibility genes *i.e.* ADAM33 (20p13[7]), PHF11 (13q14.3 [29]), DPP10 (2q14.1 [27]), HLAG (6p21.3 [26]), GPRA (7p14.3 [30]), IL1RN (2q13 [28]) and the recently reported gene ORMDL3 (17q12-q21[24]) were identified by the GSMA approach, ADAM33 (p(OR) = 0.005), DPP10 and IL1RN (p(OR) = 0.007) and less significantly HLA-G (p(SR) = 0.039) and GRPA (p(OR) = 0.031). In addition our data also suggests that further investigation of additional chromosomal regions may be productive *e.g.* 1p31.1-p13.3 and 14q32.12-qter. Recently, the 1p31 and 14q32 regions have been highlighted as potential asthma loci in a French cohort with data suggesting 1p31 may contain gene(s) of importance to asthma and atopic dermatitis co morbidity and the 14q32 locus may interact with smoking exposure and contain asthma susceptibility gene(s) [31,32].

The analysis of overlap between chromosomal regions confirmed the importance of the HLA locus on chromosome 6 as being a key susceptibility locus in asthma and also highlighted other regions that may be of importance *i.e.* 5q23.2-q34 and 7p21.1-14.1. The 5q23.2-q34 region contains the cytokine gene cluster (*IL4*, *IL13*, *IL5*, *IL12B*) and has previously been suggested as an asthma/atopy susceptibility locus [3] and the 7p21.1-14.1 region con-

tains the previously identified asthma susceptibility gene, GPRA (7p14.3) [30].

In conclusion, we present the first systematic meta-analysis of asthma and related trait linkage data in the Caucasian population. These data are based on the majority of the data available in the public domain (or through collaboration) therefore we do not consider that exclusion or missing data for other populations has biased our analyses. While the GSMA method has limitations *e.g.* only large chromosomal regions can be identified, these analyses have determined the role of several previously identified susceptibility loci and highlighted the significance of regions not previously implicated in asthma and atopy susceptibility. Importantly, this study also highlighted the limitations of using asthma as a phenotype in contrast to quantitative traits even with the increased power of 1,267 families composed of 5,832 individuals. Finally, these data will provide useful guidance for the interpretation of the anticipated genome wide association analyses in asthma and atopy.

List of abbreviations

GSMA: Genome Scan Meta-analysis; SPT: allergen positive skin prick test; IgE: total serum Immunoglobulin E (IgE); BHR: bronchial hyper-responsiveness (BHR).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IS designed the study, compiled and interpreted results and wrote the manuscript. SD contributed to the study design, completed the data analyses and contributed to the writing of the manuscript. GHK, MW and MAF provided datasets, contributed to the design of the study and the writing of the manuscript. JB and IPH contributed to the design of the study and the writing of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

I. Sayers is supported by the Medical Research Council (New Investigator Award) and the Dutch family studies were supported by The Netherlands Asthma Foundation and the National Institute of Health (NIH). We thank Professor William Cookson for providing datasets and Dr Cathryn Lewis for making the GSMA software available.

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