Human Molecular Genetics, 2001, Vol. 10, No. 8 891–899

Meta-analysis for linkage to asthma and atopy in the chromosome 5q31–33 candidate region

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Received 7 December 2000; Revised and Accepted 5 February 2001

Asthma is a common, complex human disease. Gene discovery in asthma has been complicated by substantial etiological heterogeneity, the possibility of genes of small effect and the concomitant requirement for large sample sizes. Linkage to asthma phenotypes has been investigated most intensively in the 5q chromosomal region, although results have been inconsistent across studies and all studies have had modest sample sizes. One potential solution to these issues is to combine data from multiple studies in a retrospective meta-analysis by pooling either summary statistics or raw data. The International Consortium on Asthma Genetics combined data from 11 data sets (n = 6277 subjects) to investigate evidence for linkage of 35 markers spanning the cytokine cluster on chromosome 5q31–33 to 'asthma' dichotomy and total serum immunoglobulin E (IgE) levels. Chromosome 5q markers typed in different centers were integrated into a consensus map to facilitate effective data pooling. Multipoint linkage analyses using a new Haseman–Elston method were performed with all data sets pooled together, and also separately with the resulting linkage statistics pooled by meta-analytic methods. Our results did not provide any evidence significant at the 5% level that loci conferring susceptibility to asthma or atopy are present in the 5q31–33 region; however, there was some weak evidence (empirical P = 0.077) of linkage to asthma affection. This study suggests that loci in 5q31–33 have at most a modest effect on susceptibility to asthma or total serum IgE levels, may not be detectable or present in all human populations and are difficult to detect even using combined linkage evidence from 2400–2600 full sibling pairs.

INTRODUCTION

Asthma is the most common chronic childhood disease in developed nations (1), affecting more than 155 million individuals. The cost of treating this disease in the US alone approaches six billion dollars per annum (2). Asthma is closely associated with the familial syndrome of atopy, which is accompanied by increased levels of total serum immunoglobulin E (IgE) (3). Total serum IgE levels are the best characterized intermediate phenotype associated with symptoms of asthma (3–5).

Mapping human susceptibility loci for asthma is made difficult by a high population frequency, incomplete penetrance, phenocopies, genetic heterogeneity, possible epistasis and pleiotropy (6,7); replication of any positive results may be difficult and often the significance of different findings among studies is controversial. Although significant progress has been made in defining the genetic basis of asthma and atopy over the last decade (6,7), even large studies are likely to have had low power to map genes of modest effect by linkage. This is particularly true for the phenotype 'asthma' affection (8). One potential

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solution to these issues is to combine data from multiple studies (9–11).

The Consortium on Asthma Genetics (COAG) was established in 1999 as an international effort to define the candidate regions and genes for asthma and atopy with greater precision and reliability than can be achieved by smaller individual studies. Given that one of the most investigated regions of the human genome for linkage to asthma phenotypes has been the 5q region, an initial retrospective study focusing on combination of evidence for the cytokine region on chromosome 5q was established (COAG-5). Participation by research groups was on the basis of availability of linkage data in the 5q31–33 region; there was no intentional bias for or against positive studies. This region is regarded as a biologically sensible candidate location for atopy and asthma susceptibility loci (12), as it contains a large number of important candidate genes (13) including the cytokine genes interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 9 (IL-9), interleukin 13 (IL-13) and their receptors. Other candidate genes in this region include granulocyte macrophage colony stimulating factor (GM-CSF), fibroblast growth factor acidic (FGFA) and the β_2-adrenergic receptor gene. Linkage of asthma-associated phenotypes has been reported to this region by several locus-specific studies (13–15) and whole genome screens (16–19). However, despite these positive studies, other studies have not shown linkage (20–23). All studies had modest sample sizes and some level of positive publication bias is possible.

In the current study, therefore, the evidence for linkage to asthma and atopy phenotypes in the 5q31–33 region has been investigated by combining linkage data from 11 diverse studies comprising a total of 1037 families and 6278 subjects.

RESULTS

Linkage analysis

The multipoint pedigree likelihood-based method used to check for possible genotyping errors (24) suggested that the maximal potential error rate in any one data set was <1% of all genotypes. Therefore, our error-checking procedure resulted in the exclusion of very few families at a given marker from any of the data sets.

Analysis of the among-study variance of the estimates of the pooled regression coefficients ($\hat{\beta}_i$), which reflect the location-specific linkage in each of the study populations (9), suggested that the among-study variability of the coefficients was, if anything, smaller than would be expected by chance. There was therefore no reason to suspect that the linkage model was different in the different studies, and hence the among-study variance term could safely be set to zero in these meta-analyses. The residual errors from the linkage regressions for asthma affection varied significantly ($P < 0.01$) from study to study (i.e. were heteroscedastic) using either Cochran's C test ($P = 0.14$) or Hartley's F_{max} test ($P = 4.0$) (25). Conversely, the residual errors from the linkage regressions for ln(total IgE) levels varied little from study to study (i.e. were homoscedastic) using Bartlett's test ($P > 0.05$) (25).

The results of the linkage analyses for asthma and ln(total IgE) levels adjusted for age and sex are shown for each separate study population in Figure 1. The Oxfordshire and Southampton populations showed some evidence ($0.01 <$ empirical $P < 0.05$) of linkage to asthma in the proximal part of this chromosomal region (Fig. 1). The Collaborative Study on the Genetics of Asthma African-American and the Chinese samples showed similar evidence of linkage to markers in the distal part of the region (Fig. 1). The remaining study populations showed little evidence of significant linkage to asthma across the 5q31–33 region. The Barbados and Finnish populations both showed some evidence ($0.01 <$ empirical $P < 0.05$) to ln(total IgE) levels in the proximal part of the region. The remaining study populations showed no evidence of significant linkages to ln(total IgE) levels across the 5q31–33 region.

The results of the pooled linkage analyses of the dichotomous 'asthma' phenotype and the ln(total IgE) are shown in Figure 2. For the dichotomous asthma phenotype, 2573 full sibling pairs were informative. For ln(total IgE), 2416 full sibling pairs were informative. No evidence for linkage (significant at the 0.05 level) with either of the phenotypes was found when the data sets were combined and analyzed together, or when linkage evidence was pooled either by weighting by the number of pairs contributed by each data set [method W1] or by the inverse variance of the estimated regression parameter [method W2] (Fig. 2). All empirical P values were ≥0.077 for both phenotypes. Evidence of linkage to the dichotomous asthma phenotype using weights W1 was maximal over an ~6 cM region between D5S421 and D5S404, and peaked at interpolated position 144.75 cM from pter (empirical $P = 0.077$, $t = 1.428$, 2194 full sibling pairs). The regression coefficient at this position (the variance in the trait due to a trait locus, if any, in this position) explained 2.16% of the variance of asthma dichotomy after adjustment for age and sex. Additional analyses of asthma affection using an all-affected-relative-pairs linkage test (26) in the pooled data set gave consistent results: evidence of linkage was maximal at D5S2501 (LOD = 1.58, using 1133 affected relative pairs). The evidence of linkage to ln(total IgE) was maximal using the pooled raw data at marker D5S1505 (empirical $P = 0.126$; $t = 1.145$; 2416 full sibling pairs).

Identical multipoint analyses using simpler Haseman–Elston models (27) were consistent with the linkage results presented in Figures 1 and 2 (data not shown), although they had less statistical power (28,29).

DISCUSSION

This study was designed to investigate the evidence for linkage to asthma phenotypes in the 5q31–33 region in a retrospective analysis of combined data from 11 separate studies. Our results did not reach conventional significance levels ($P < 0.05$) for any phenotype using any method, although our meta-analysis suggested that there was some weak evidence of linkage (empirical $P = 0.077$) to asthma in the proximal part (D5S2501–D5S622) of the 5q31–33 region. Our results were consistent with the conclusions that loci in 5q31–33 have at most a modest effect on susceptibility to asthma or total serum IgE levels, may not be detectable or present in all human populations and are difficult to detect even using combined linkage evidence from 2400 to 2600 full sibling pairs.

Previous studies, both positive and negative, had modest sample sizes and individual studies may have involved low power or small effects or both. The evidence for linkage between asthma and/or atopy and 5q markers has been variable amongst published studies, and most of the positive linkage

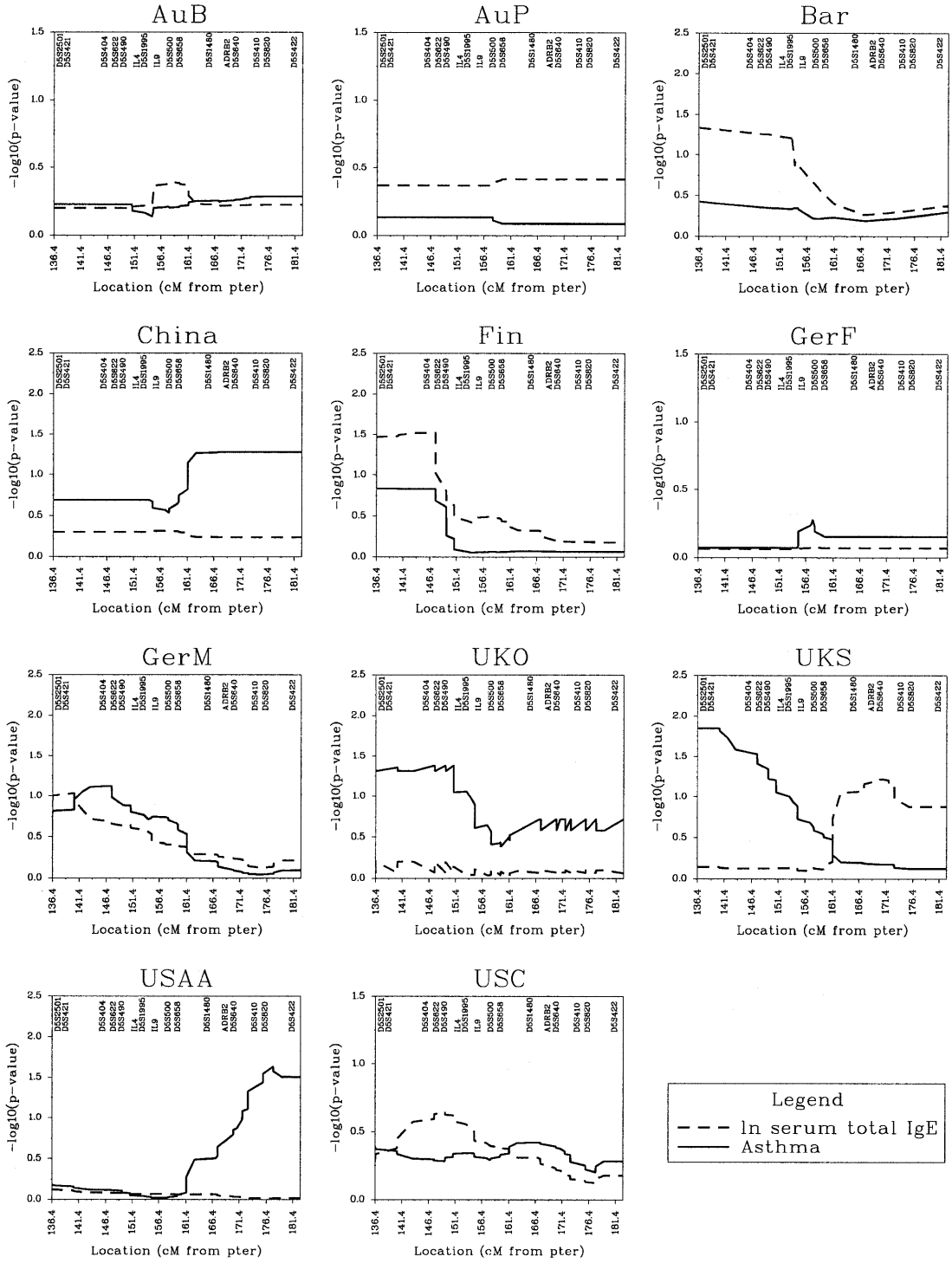

Figure 1. Linkage of 5q markers to asthma and ln(total IgE) levels in individual data sets.

studies showed only modest evidence of linkage (7). Whereas some studies show significant evidence of linkage of asthma and/or total IgE levels to 5q31–33 (Fig. 1), not all previous studies of chromosome 5q31–33 may have been large enough to detect modest effects or to exclude the region from further consideration. The results from our analyses regarding individual studies suggested no obvious selective bias for or against positive studies; around half of the studies yielded some evidence of linkage (Fig. 1).

A primary concern when combining data from diverse populations is the potential inter-population heterogeneity in study design, phenotype definition, genetic structure, environmental exposures and markers typed. The current study implements a number of strategies for dealing with these issues: the ln(total IgE) phenotype was standardized within each study/ethnic group;

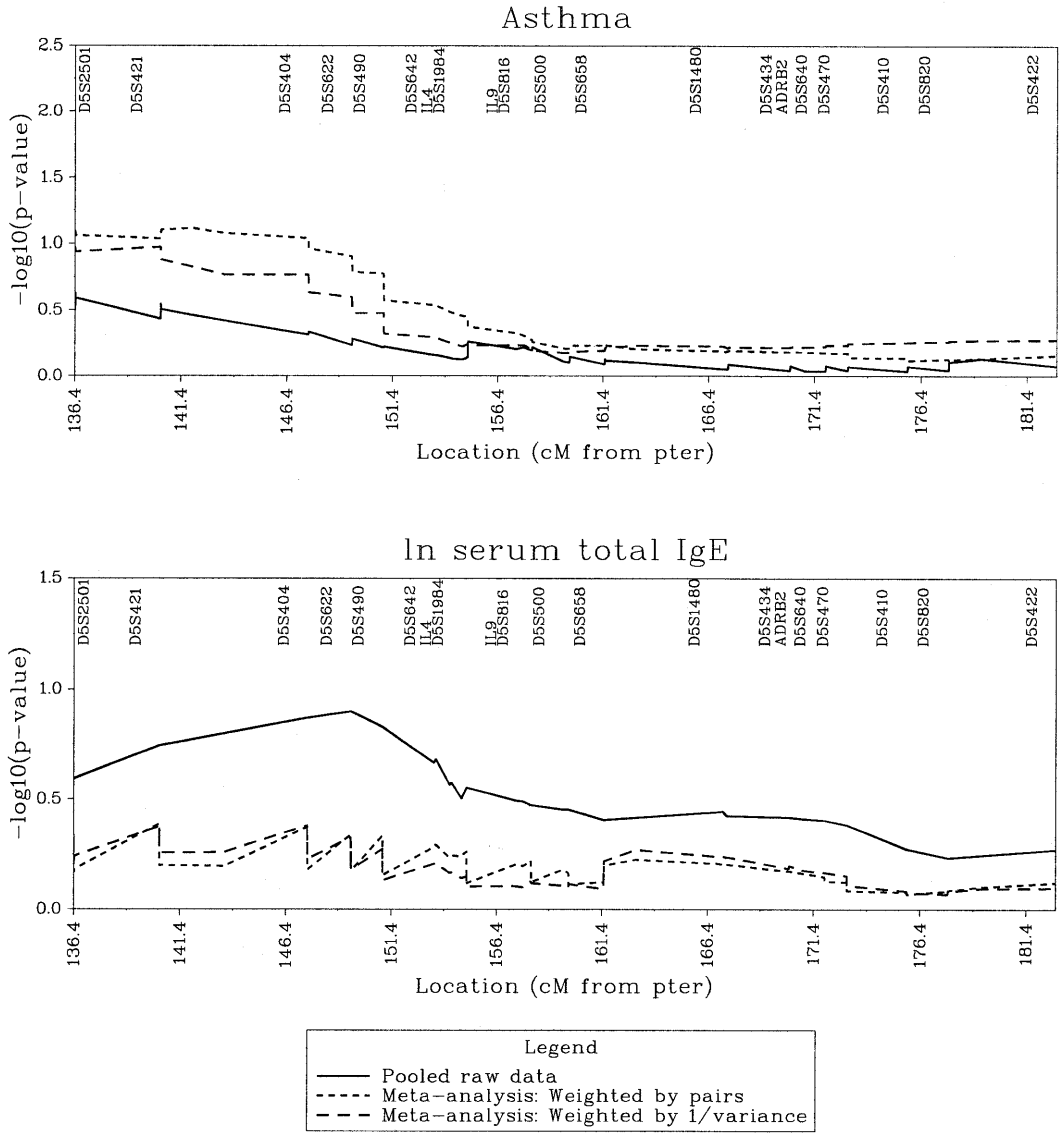

Figure 2. Linkage of 5q markers to asthma and ln(total IgE) levels in pooled data and combined study-specific linkage statistics.

the same 'consensus' genetic map derived from well-validated public databases was used in each study population and in the overall pooled data analyses, for uniform multipoint linkage and interpolation at untyped locations; a new and more powerful Haseman–Elston linkage test was used; *P* values were generated empirically; and several data pooling and meta-analytic strategies were applied. Extrapolation of allele sharing idendical-by-discent (i.b.d.) in some places could have led to less precise estimates of linkage. However, the meta-analysis weighting method, W2, appropriately accounted for the differences in precision of estimates at different points across the 5q31–33 region among studies. If one or more common genes linked to asthma susceptibility or total IgE levels were present in this region in the combined study populations, this combination of analyses should probably have found some evidence of these loci.

An important advantage of our study was that the COAG retrospective collaboration allowed us to pool raw data across data sets, which, in combination with multipoint procedures, is an obvious and probably optimal method for combining linkage evidence from multiple studies (30–32). Weighting our meta-analysis by either the inverse of the variance of the regression coefficient (9) or by the number of pairs gave consistent results, suggesting that our analysis was robust to the implicit assumptions of the meta-analyses.

The most optimistic interpretation of our analyses is that they suggest the possible presence of susceptibility loci for asthma having modest effects (explaining <5% of total variance) in the proximal area of the 5q31–33 region. The genes for IL-4 and IL-13 are in this area. Less evidence of linkage to total IgE levels was found. If one or more loci that have an important effect on asthma susceptibility or total IgE levels in multiple study populations do exist in the 5q31–33 chromosomal region, there are several reasons why our pooled analyses may not have found them. The effect size of any susceptibility gene(s) may have been below the limit of the size detectable even in a combined sample of 2400–2600 sibling pairs. This in turn could imply gene(s) of quite minor effect and/or low

Table 1. Details of study populations

Centre	References	No. families[a]			No. markers typed in 5q31–33 region	Code
		r	m	p		
Busselton, Australia	20	80	–	–	10	AuB
Perth, Australia	41	98	–	25	2	AuP
Barbados	42	–	33	–	5	Bar
Fujian, China	43	–	9	23	3	China
Finland	21	–	39	15	16	Fin
Munchen, Germany	22	–	97	–	9	GerM
Freiburg, Germany	44	–	–	72[b]	5	GerF
Oxfordshire, UK	45	–	–	80	8	UKO
Southampton, UK	46	131	60	49	13	UKS
US African-American	16	–	114	–	5	USAA
US Caucasian	16	–	112	–	5	USC

[a]r, nuclear family unselected with regard to asthma or atopy; m, extended pedigree selected on multiple individuals with asthma; p, nuclear family selected on the basis of at least one child having asthma.
[b]Selection on atopic proband.

Table 2. Characteristics of sibling pairs in study populations

Population code	Total sibling pairs (*n*)	Asthma definition[a]	Asthma (%)	Male sex (%)	Age (years) (SD)	Total serum IgE (IU/ml) (95% CI)[b]
AuB	164	Q1	17.3	51.2	12.6 (4.3)	55.5 (44.1–69.8)
AuP (random)	166	Q1	37.3	52.9	6.7 (3.7)	65.6 (52.9–81.4)
AuP (ascertained)	96	Q1	69.5	43.9	11.9 (6.4)	153.2 (105.6–222.4)
Bar	363	Q4	33.8	44.7	23.0 (14.2)	1024.2 (872.7–1202.1)
China	43	Q4	73.3	56.7	10.4 (8.2)	166.2 (135.8– 203.3)
Fin	58	Q2	72.9	40.7	34.5 (18.4)	82.0 (61.7–109.0)
GerM	155	Q3	90.5	56.6	11.0 (4.2)	177.0 (141.5–221.3)
GerF	229	Q1	21.05	50.1	14.8 (4.7)	137.0 (112.4–167.0)
UKO	179	Q1	52.4	54.0	18.2 (10.1)	136.7 (86.4–216.3)
UKS (random)	444	Q1	19.8	48.7	13.6 (4.2)	73.1 (61.0–87.6)
UKS (ascertained)	187	Q1	65.4	45.6	15.1 (9.3)	91.0 (73.2–113.1)
USAA	105	C	74.9	51.1	16.7 (10.2)	181.6 (155.6–211.8)
USC	394	C	69.5	47.4	21.5 (13.7)	106.2 (89.9–125.4)

[a]Q1, questionnaire definition (physician diagnosed asthma ever); Q2, questionnaire definition (self-reported history of asthma); Q3, questionnaire definition (physician diagnosed asthma ever) plus a history of at least 3 years of recurrent wheezing and with no other airway diseases; Q4, questionnaire definition (self-reported history of asthma) plus physician exam (current symptomatic asthma); C, compound definition, at least two symptoms (cough, wheeze, dyspnea) and either (i) a positive response to methocholine challenge by the end of challenge or (ii) a ≥15% increase in baseline FEV_1 after bronchodilator use.
[b]Geometric mean and 95% confidence intervals (±1.96 SEM).

frequency. Alternatively, genetic heterogeneity involving multiple susceptibility loci in the 5q31–33 region could have negatively affected the power of our study to detect linkage. Other possible reasons include differences between studies in the definition of asthma and/or marker density (Tables 2 and 3), the presence of epistatic interactions, gene–environment interactions and/or substantial inter-population genetic and environmental heterogeneity. Finally, it should be noted that several studies that have previously reported positive linkage of asthma or atopy to this region were not available for this meta-analysis (14,17,33).

Our results regarding total IgE levels were consistent with a contemporaneous meta-analysis of the same data that used different statistical methods to combine LOD scores calculated at fixed locations within each data set using data set-specific genetic maps (34). However, in contrast to our study, their meta-analysis of asthmatic sibling pairs found significant evidence of linkage to asthma in the proximal area of the

Table 3. Concensus genetic map and marker order used in linkage analyses

Locus	Mb	Male cM	Female cM	Average cM	Band	Study population										
						AuB	AuP	Bar	China	Fin	GerM	GerF	UKO	UKS	USAA	USC
ptr	0	0	0	0	p15.33	–	–	–	–	–	–	–	–	–	–	–
D5S2501	122.058	109.49	163.26	136.38	q22.3	–	–	–	–	–	–	–	–	–	+	+
D5S421	124.957	110.94	169.91	140.43	q23.1	–	–	–	–	–	+	–	–	+	–	–
D5S404	133.19	114.6	180.24	147.42	q23.3	–	–	–	–	+	+	–	–	–	–	–
D5S622	135.548	115.95	182.97	149.46	q23.3	–	–	–	–	+	+	–	–	–	–	–
D5S1505	135.564	115.96	182.97	149.47	q23.3	–	–	–	–	–	–	–	–	–	+	+
D5S490	136.85	117.2	184.72	150.96	q23.3	+	–	–	–	+	–	–	+	–	–	–
D5S642	138.24	119.23	187.56	153.4	q23.3	–	–	+	–	+	–	–	–	+	–	–
D5S808	138.347	119.41	187.57	153.49	q23.3	–	–	+	–	–	–	–	–	–	–	–
D5S666	139.18	120.56	187.74	154.15	q31.1	–	–	+	–	–	–	–	–	+	–	–
IL4	139.35	120.56	187.9	154.23	q31.1	+	–	+	+	+	+	+	+	+	–	–
D5S1984	140.372	120.58	188.84	154.71	q31.1	+	–	–	–	–	–	–	+	–	–	–
IRF1	140.43	120.58	188.89	154.73	q31.1	–	–	–	–	+	–	+	–	–	–	–
D5S1995	140.711	120.59	189.15	154.87	q31.1	–	–	–	–	+	–	–	–	–	–	–
D5S2117	140.88	120.59	189.31	154.95	q31.1	+	–	–	–	–	–	–	+	–	–	–
D5S2115	142.43	121.84	192.68	157.26	q31.1	–	–	–	–	+	–	–	–	–	–	–
IL9	142.648	121.88	193.41	157.64	q31.1	+	–	–	–	+	+	+	+	+	–	–
D5S816	142.743	121.9	193.72	157.81	q31.1	–	–	–	–	+	–	–	–	–	+	+
D5S393	142.86	121.93	194.11	158.02	q31.1	+	+	–	+	+	–	+	–	+	–	–
D5S500	144.453	122.62	196.46	159.54	q31.2	–	–	–	–	+	–	–	+	–	–	–
D5S414	144.64	122.63	196.6	159.62	q31.2	–	–	–	–	–	+	–	–	–	–	–
D5S399	144.876	122.7	196.9	159.8	q31.2	+	+	–	–	+	–	+	–	+	–	–
D5S658	147.01	123.38	199.57	161.47	q31.2	–	–	–	–	–	–	–	–	+	–	–
D5S1480	151.313	125.31	208.47	166.89	q31.3	–	–	–	–	–	–	–	–	–	+	+
D5S436	152.5	125.36	208.84	167.1	q31.3	+	–	+	+	+	–	–	+	+	–	–
D5S210	152.609	125.44	209.15	167.3	q31.3	+	–	–	–	–	–	–	–	+	–	–
D5S434	155.051	126.52	213.91	170.22	q32	–	–	–	–	+	–	–	–	–	–	–
D5S413	156.38	126.65	215.24	170.94	q32	–	–	–	–	+	–	–	–	–	–	–
ADRB2	156.38	126.65	215.24	170.94	q32	–	–	–	–	–	–	–	–	+	–	–
D5S640	158.081	126.83	216.95	171.89	q32	–	–	–	–	–	+	–	–	–	–	–
CSF1R	158.151	126.83	217.02	171.93	q32	–	–	–	–	–	–	–	+	–	–	–
D5S470	159.849	127.02	218.85	172.94	q33.1	–	–	–	–	–	–	–	–	+	–	–
D5S410	163.279	127.5	223.98	175.74	q33.1	+	–	–	–	–	+	–	–	+	–	–
D5S820	166.497	130.18	225.18	177.68	q33.3	–	–	–	–	–	–	–	–	–	+	+
IL12B	171.59	134.3	227.98	181.14	q34	–	–	–	–	–	–	–	–	–	–	–
D5S422	174.37	135.74	229.83	182.79	q34	–	–	–	–	–	+	–	–	–	–	–

cytokine cluster, around reference locus D5S1505 (LOD = 2.61, $P = 0.0025$) (34). The difference in linkage results for asthma may be due to the use of differing statistical techniques, to the use of differing definitions for asthma and atopy, including those derived from a principal components analysis, and to the omission of two large negative studies (the AuB and GerF samples) from the primary analysis of affected sibling pairs by Lonjou *et al.* (34). Other potentially important differences between these studies include the use of empirical versus asymptotic *P* values, the use of genetic maps generated within

each study population versus the use of a consensus map and differing approaches for dealing with potential genotyping errors.

Several well-designed SNP association studies have provided evidence that polymorphisms in the *IL-13* and *IL-4* genes in the cytokine cluster of 5q31–33 regulate serum IgE levels (35–38). However, the largest of these studies (37) found that polymorphism in the *IL-13* gene, while strongly associated with total IgE levels, explained only 1.6% of the variation in this trait. Together with our results, these data are consistent with the argument that association analysis is likely to be far more powerful than linkage for detecting susceptibility loci (which may be of small effect) for common, complex diseases (39,40).

Conclusions

This study has provided a new perspective on the potential significance of the 5q31–33 region in asthma genetics for the phenotypes studied. Meta-analysis is an emerging methodology in complex disease genetics; the combination of evidence from multiple studies can be expected to be critical to the successful localization of genes of modest effect for any common, complex disease such as asthma. This study also illustrates the benefits of cooperation between groups studying a complex human disease, that data from diverse studies and populations can be effectively pooled and that new and important information can result from the analysis of large, pooled data sets. It is hoped that further research and methodological development, together with growing international participation in COAG, will lead to major advances in our understanding of the genetic basis of asthma.

SUBJECTS AND METHODS

Study subjects and phenotypic data

A total of 1037 pedigrees, consisting of 1561 nuclear families, 6278 individuals and 2583 sibling pairs were available for analysis. Data came from Australian (20,41), Barbadian (42), Chinese (43), Finnish (21), German (22,44), British (45,46) and US Caucasian and African-American (16) populations (Table 1). Data from these 11 studies (Tables 1 and 2) were analyzed for linkage. Two data sets (41,46) contained a subset of families that were ascertained for asthma or atopy as well as a subset unselected with regard to asthma or atopy. Thus, the data comprised three random population samples and 10 that were selected for asthma or atopy (Tables 1 and 2). A total of 35 markers from the chromosome 5q region were analyzed, although differing numbers of markers were typed in each individual study (Table 3).

The primary response variables, which were available in all 11 studies, were the dichotomous trait 'asthma affection', defined in varying ways in the 11 studies (Table 2), and total serum IgE levels, measured by standard ELISA techniques (Pharmacia AB).

Total serum IgE levels were positively skewed and were $\log_e$ transformed and converted to Z-scores prior to analysis, i.e. they were standardized to a mean of 0 and a standard deviation of 1 within each data set and ethnic group. Sex, age and age × sex interaction were allowed for as covariates in the statistical analyses.

All study populations were collected under appropriate institutional ethics approvals, as detailed in the original papers (referenced in Table 1).

Consensus map

A consensus map of chromosome 5q31–33 that incorporated all typed markers in each of the 11 data sets was constructed to pool data across samples (47) (http://cedar.genetics.soton.ac.uk/public.html/). This map provided a framework of markers upon which typed markers on chromosome 5q were integrated; marker order was validated by consulting physical maps (http://www-shgc.stanford.edu/).

Linkage analysis

The GENIBD program (http://darwin.cwru.edu/pub/sage.html) was used to estimate multipoint allele sharing i.b.d. at 58 locations spanning the cytokine cluster on chromosome 5q31–33 from D5S5201 to D5S5422 using a variety of techniques (48–51). These locations consisted of the 35 markers typed across the 11 data sets and 23 points spaced at ~2 cM intervals between markers. Since each data set contained a different subset of typed markers (Table 3), allele sharing i.b.d. was interpolated or extrapolated when necessary using all available marker data.

Linkage analysis was performed on the phenotypes of interest and the estimated allele sharing i.b.d. using the SIBPAL2 (version 4.0 beta 6) program (http://darwin.cwru.edu/pub/sage.html), which implements an enhanced multipoint version of the Haseman–Elston (27) linkage method. The new test uses an improved weighted generalized least squares procedure to obtain a uniformly more powerful procedure for detecting linkage (28,29,52,53). As a consistency check, both simpler Haseman–Elston linkage analyses based only on the squared trait difference (27) and a full conditional linkage test using all-affected-relative-pairs (26) were also performed.

A Monte Carlo permutation test was used to obtain empirical estimates of significance. After permuting i.b.d sharing estimates among sibling pairs within each data set, each replicate was analyzed using an identical model to that used in the original analysis.

Error checking. To detect likely genotyping errors, we calculated the multipoint pedigree likelihood for all available genotypes (54). Single genotypes that implied a large number of recombination events (and induced an unexpectedly large change in the likelihood, greater than a factor of 100) were flagged as errors and the entire family containing that individual removed from the analysis for that marker.

Combination of evidence for linkage

Two methods for combining linkage evidence across data sets (meta-analysis), pooling raw data and pooling data set-specific linkage statistics, were investigated. Pooling raw data assumes that there is some degree of homogeneity among populations, although additional analyses that minimized the effect of this assumption (e.g. by explicitly allowing polygenic and residual phenotypic variability to differ from study to study; 55)

confirmed our primary conclusions (data not shown). The second method is robust to heterogeneity among data sets, and allows all estimated parameter values to differ among data sets.

In order to perform a meta-analysis of the linkage results within each study population, two different weighted least squares estimates $\hat{\beta}$ of the pooled location-specific regression coefficients $\hat{\beta}_i$ from each of the n study populations were used to obtain an overall test statistic at each location:

$$\hat{\beta} = \frac{\Sigma_{i=1}^{n} w_i \hat{\beta}_i}{\Sigma_{1=1}^{n} w_i};$$

$\hat{\beta}$ divided by the square root of its estimated variance was then referred to the standardized normal (Z) distribution (this assumes that we have a large number of degrees of freedom and that these $\hat{\beta}_i$ are uncorrelated, i.e. that the $\hat{\beta}_i$ are estimated from independent samples, which they were). Two different weighting methods were used in the pooling of the location-specific linkage coefficients across data sets. Both weighted the location-specific coefficients in a manner that reflected the amount of information upon which each was based. Importantly, neither weighting structure would have systematically biased the pooled test statistic for or against linkage under the null hypothesis. Method W1 weighted the linkage evidence from each data set by the number of full sibling pairs in the *i*th data set. Method W2 weighted evidence proportionally to the inverse of the variance of each regression coefficient, i.e. coefficients that had been estimated precisely received more weight than those that had been estimated less precisely (9). In each case the variance includes a random effect for among-study variation to allow for heterogeneity.
Formally:

$$[\mathbf{W1}]\ w_{1i} = n_i$$

and

$$[\mathbf{W2}]\ w_{2i} = \frac{1}{\sigma^2 + \hat{S}_i^2}$$

where n_i is the number of informative full sibling pairs from the *i*th data set, $\hat{S}_i^2$ is the estimated variance of $\hat{\beta}_i$ estimated from each linkage study and σ^2 is the among-study variance of $\hat{\beta}_i$ (9). With the weights W1 the variance of $\hat{\beta}_i$ is

$$\frac{\Sigma_{i=1}^{n}(n_i^2(\sigma^2 + \hat{S}_i^2))}{(\Sigma_{i=1}^{n} n_i)^2}$$

whereas with weights W2 it is

$$\frac{1}{\Sigma_{i=1}^{n} w_{2i}}$$

ACKNOWLEDGEMENTS

We are grateful to Professor Newton Morton for his generous contribution of data to this paper. COAG was founded under a grant from the National Asthma Campaign, UK and has received ongoing support from the Medical Research Council, UK. Some of the methodological work used in the analysis was supported by Program Grant 00\3209 from the National Health and Medical Research Council of Australia. Some of the results in this paper were obtained by using the program package S.A.G.E., which is supported by a US Public Health Resource Grant (1 P41 RR03655) from the National Center for Research Resources. During the work, Dr Lyle Palmer held Fulbright and Churchill Fellowships, and was a National Health and Medical Research Council of Australia Postdoctoral Research Fellow. Many people in 14 research groups contributed to this collaboration. They and the grants that supported their work are cited in the references given in Table 1.

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