

A genome-wide association study to identify genetic determinants of atopy in subjects from the United Kingdom

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Background: A genetic component in the development of atopy has been identified. However, numerous heritability models have been proposed with inconsistent replication of susceptibility loci and genes.

Objective: We sought to use a genome-wide association study approach to examine genetic susceptibility to atopy, which was defined as increased specific IgE levels, positive skin prick test (SPT) responses, or both, within a large discovery cohort and 3 additional white populations.

Methods: Single nucleotide polymorphisms (SNPs) across the genome were tested for association with increased specific IgE levels (≥ 0.35 kU_A/L) in the British 1958 Birth Cohort (1083 cases and 2770 control subjects; Illumina 550K Array) to 1 or more allergens, including house dust mite (Der p 1), mixed grass, or cat fur. Independent replication of identified loci

($P \leq .05$) was assessed in 3 case-control cohorts from the United Kingdom ($n = 3225$). Combined analyses of data for top signals across cohorts were conducted for atopic phenotypes: increased specific IgE levels (1378 cases and 3151 control subjects) and positive SPT responses (1058 cases and 2167 control subjects). **Results:** A single SNP on chromosome 13q14 met genome-wide significance ($P = 2.15 \times 10^{-9}$), and a further 6 loci ($4.50 \times 10^{-7} \leq P \leq 5.00 \times 10^{-5}$) showed weaker evidence for association with increased specific IgE levels in the British 1958 Birth Cohort. However, no SNPs studied showed consistent association with atopy defined by increased specific IgE levels, positive SPT responses, or both in all study cohorts.

Conclusions: Seven putative atopy loci were identified using a genome-wide association study approach but showed limited replication across several white populations. This study suggests that large-scale analyses with results from multiple populations will be needed to reliably identify key genetic factors underlying atopy predisposition. (J Allergy Clin Immunol 2010;■■■■:■■■-■■■.)

Key Words: Atopy, specific IgE, skin prick test, genome-wide association study, British 1958 Birth Cohort

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We acknowledge use of phenotype and genotype data from the British 1958 Birth Cohort DNA collection, funded by Medical Research Council grant G0000934 and Wellcome Trust grant 068545/Z/02 (<http://www.b58cgenome.sgul.ac.uk/>). This analysis uses genotype data deposited by the Wellcome Trust Sanger Institute and by the Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research, University of Cambridge, which is funded by the Juvenile Diabetes Research Foundation International, the Wellcome Trust, and the National Institute for Health Research Cambridge Biomedical Research Centre; the CIMR is in receipt of a Wellcome Trust Strategic Award (079895). The British 1958 Birth Cohort genotypes deposited by DIL were generated by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute of Allergy and Infectious Diseases (NIAID), the National Human Genome Research Institute (NHGRI), the National Institute of Child Health and Human Development (NICHD), and the Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418. UK Medical Research Council, the Wellcome Trust, and the University of Bristol provide core support for the Avon Longitudinal Study of Parents and Children Cohort. The Nottingham Gedling cohort collection was funded by Asthma UK and the British Lung Foundation. The Southampton cohort is supported by Asthma, Allergy, and Inflammation Research (AAIR). Disclosure of potential conflict of interest: J. Henderson receives research support from the Medical Research Council (UK). I. P. Hall receives research support from Asthma UK. The rest of the authors have declared that they have no conflict of interest.

Received for publication May 27, 2010; revised October 5, 2010; accepted for publication October 8, 2010.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2010.10.006

Atopy is the predisposition to increased IgE isotype production and the development of immediate hypersensitivity in response to exposure to common environmental allergens.¹ In genetic epidemiologic studies atopy has generally been defined either by increased specific IgE levels or positive skin prick test (SPT) responses. In addition, the majority of subjects with increased specific IgE levels also have increased total IgE levels.²

The production of allergen-specific IgE is one of the strongest risk factors for the development of allergic diseases, such as asthma, allergic rhinitis, and atopic dermatitis.^{3,4} It has been demonstrated that 60% of asthmatic subjects show immediate hypersensitivity to defined environmental allergens, and the prevalence of other atopic diseases, such as allergic rhinitis and atopic dermatitis, is also increased in asthmatic subjects.⁵

A genetic component in the development of allergic diseases has been recognized since 1916, when a positive family history was reported in almost 50% of patients with asthma and allergic rhinitis.⁶ Genetic susceptibility to atopy has been confirmed by numerous epidemiologic studies showing familial clustering of disease and high concordance rates among twins.⁷

Estimates of heritability vary, ranging from 34% for the RAST index, 35% for positive SPT responses, and 47% to 74% for serum total IgE levels.^{8,9} Multiple chromosomal regions, including loci on 2q, 5q, and 11q, have been reported to show some evidence of linkage to atopy and have been suggested to contain disease susceptibility genes.¹⁰

Abbreviations used

ALSPAC: Avon Longitudinal Study of Parents and Children
 FNDC3A: Fibronectin type III domain containing protein
 GWAS: Genome-wide association study
 SNP: Single nucleotide polymorphism
 SPT: Skin prick test

We previously reported a meta-analysis of 11 genome-wide linkage studies in the white population. Significant evidence for linkage was shown for chromosomal regions 3p22.1-q22.1 and 17p12-q24.3, suggesting they might contain susceptibility loci for positive SPT responses, at least in asthma-enriched families (1093 pedigrees, $n = 4746$).¹¹

Polymorphisms within several candidate genes identified through positional and functional approaches, such as interleukin 4, interleukin 4 receptor, interleukin 13, major histocompatibility complex, class II, DR beta 1, and Beta-chain of the high-affinity receptor for IgE, have also shown reproducible association with atopy and related phenotypes.¹² In addition, recent genome-wide association studies (GWASs) have identified genes including *ORMDL3* for childhood asthma susceptibility and *FCERIA* for serum total IgE levels.^{13,14} However, no study using a genome-wide approach has examined predisposition to atopy *per se*.

Previous research with subjects from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort has shown that 70% of all atopic children were identified from their reactions to 3 common allergens alone: house dust mite, mixed grass pollen, and cat fur.¹⁵ The aims of the current study were to conduct a large-scale GWAS examining atopy defined by increased levels of specific IgE to house dust mite, mixed grass, or cat fur in 3853 white adults from the British 1958 Birth Cohort and to replicate the top signals in a total of 3225 samples from 3 independent cohorts from the United Kingdom.

METHODS**Study population and procedures**

The primary GWAS was carried out in the British 1958 Birth Cohort. Replication of loci identified for association was performed in 3 independent case-control cohorts using both *in silico* replication (directly genotyped or imputed) and prospective genotyping. Informed consent was given by all study participants, and all cohort protocols were approved by local ethics committees. Demographic data for these population cohorts are summarized in Table I.

Discovery cohort. The British 1958 Birth Cohort is a longitudinal study of 18,558 persons born during 1 week in March 1958 across the United Kingdom. Follow-up at ages 7, 11, 16, 23, 33, and 42 years was conducted by using parental interviews, school medical examinations, and individual interviews.¹⁶ DNA was obtained from 7,703 singletons of white ethnicity during 2002–2004.¹⁷ Phenotype data at age 44 to 45 years were collected for atopy, as defined by increased specific IgE levels of 0.35 kU_A/L or greater to 1 or more allergens, including house dust mite (Der p 1), mixed grass, or cat fur, measured with Hytec enzyme immunoassay (Hycor Biomedical, Irvine, Calif). Genotype data were available for 1083 cases with increased specific IgE levels and 2770 control subjects, as well as (Ln) serum total IgE levels at age 44 to 45 years in 3873 subjects (Illumina 550K Array; Illumina, San Diego, Calif).

In silico replication cohorts. Single nucleotide polymorphism (SNP) data across candidate regions were available for analysis from ALSPAC. The ALSPAC dataset consisted of 282 cases and 1042 control subjects with genotype data obtained by using the Illumina 370K Array. Cases were defined by positive SPT responses with a mean wheal diameter of 2 mm or greater in response to 1 or more allergens, including house dust mite, mixed

grass, and cat, at the age of 7.5 years. Serum total IgE data were available for 1229 subjects.¹⁸

Prospective genotyping cohorts. Prospective genotyping of selected SNPs was carried out in the general population sample from the Nottingham Gedling cohort consisting of 385 cases and 822 control subjects. Cases were defined by using positive SPT responses with a mean skin wheal diameter of 3 mm or greater compared with that elicited by the saline control to 1 or more allergens, including house dust mite, cat fur, and grass pollen solutions (Bencard, London, United Kingdom). Serum total IgE data were available for 1293 subjects.¹⁹

Data from the Southampton cohort were collected from 757 nonrelated adults chosen from asthma-enriched families. Phenotype data collected for atopy were defined by serum specific IgE levels of greater than 0.35 kU_A/L determined by using RASTs for 295 cases and 381 control subjects. Three hundred eighty-two cases and 294 control subjects were identified for positive SPT responses with a wheal diameter of greater than 3 mm to 1 or more allergens, including house dust mite, grass, cat, dog, *Alternaria* species, and tree allergens. Serum total IgE levels were measured in 675 subjects.²⁰

Statistical analysis

SNPs were assessed to exclude those with minor allele frequencies of 5% or less or P values of .05 or less by using the 1 df χ^2 test for deviation from Hardy-Weinberg equilibrium in MS Excel 2007 (Microsoft, Redmond, Wash). Estimations of study power were calculated by using QUANTO version 1.2.4 software.²¹

Quality control

In the British 1958 Birth Cohort, a total of 459,334 autosomal SNPs passed all quality control measures. All SNPs taken into replication studies passed quality control checks for minor allele frequency and Hardy-Weinberg equilibrium. Minor allele frequencies of key SNPs were similar between cohorts for all SNPs apart from rs10514054 and rs6082335 (Table II).

Discovery GWAS. Nonparametric testing of all SNPs across the genome for association with increased specific IgE levels in the British 1958 Birth Cohort was conducted by using 1 df χ^2 per-allele tests comparing allele frequencies in cases and control subjects. Genome-wide statistical significance was defined by using a Bonferroni-corrected P value of 1.09×10^{-7} or less for a total of 459,334 SNPs. In addition, 2 less conservative thresholds were used to select other regions potentially containing genes of interest: a nominal P value for any single SNP of 10^{-5} or less and clustering of 3 or more SNPs with a P value of 10^{-4} or less within a 100-kb region. Loci were selected for replication analyses by setting limits of ± 300 kb flanking all identified SNPs.

In silico replication. The ALSPAC data were taken from a GWAS that has been previously described in detail.²² Briefly, 1543 ALSPAC subjects were genotyped with the Illumina HumanHap317K SNP chip. Markers with minor allele frequencies of less than 1%, SNPs with more than 5% missing genotypes, and any marker that failed an exact test of Hardy-Weinberg equilibrium ($P < 5 \times 10^{-7}$) were excluded from further analyses. After data cleaning, 315,807 SNPs were left in the ALSPAC genome-wide association analysis. Similarly, subjects of non-European ancestry or who displayed high levels of missing genotypes or heterozygosity were also excluded. This study included GWAS data for 1324 subjects with skin prick and 1423 subjects with IgE level measurements. Imputation of nongenotyped SNPs was undertaken with MACH 1.0 software.²³

χ^2 Per-allele tests were carried out for all SNPs within loci defined in the discovery cohort to test for association with positive SPT responses in ALSPAC.

Replication genotyping. SNPs showing the highest statistical significance within each locus across cohorts were prospectively genotyped in the Nottingham Gedling and Southampton cohorts. All genotyping was carried out by KBiosciences (Hitchin, United Kingdom) with KASPar single plex technology.

Association with increased specific IgE levels in the Southampton cohort and positive SPT responses in both the Nottingham Gedling and Southampton cohorts were tested by using 1 df χ^2 per-allele models. Linear regression

TABLE I. Study populations

Study	No. total	No. male	No. female	Mean age (y [SD])	Median total IgE (kU _A /L [range])	Atopy marker	No. of cases	No. of control subjects	SNP array
Discovery cohort									
B58C	3853	1889	1964	45.2 (0.4)	27 (0-1281)	Increased specific IgE level	1083	2770	Illumina 550K
Replication <i>in silico</i>									
ALSPAC	1324	642	682	7.5	22 (0.7-99.8)	Positive SPT response	282	1042	Illumina 370K
Replication genotyping									
Nottingham	1268	635	633	56.2 (12.3)	22.9 (0-2000)	Positive SPT response	394	831	KBiosciences
Gedling									
Southampton	682	339	343	42.3	9.7 (0-39,893.5)	Increased specific IgE level	295	381	KBiosciences
						Positive SPT response	382	294	
Total no. available for analysis									
Increased specific IgE level	4529						1378	3151	
Positive SPT response	3225						1058	2167	
Serum total IgE level	7276								

Characteristics are shown for cohorts analyzed in the discovery GWAS: the British 1958 Birth Cohort (B58C), replication with an *in silico* dataset (ALSPAC), and replication with prospective genotyping (Nottingham Gedling and Southampton cohorts). Atopy was defined by an increased specific IgE level (≥ 0.35 kU_A/L) in the British 1958 Birth Cohort, a positive SPT wheal diameter of 2 mm or greater in ALSPAC, a positive SPT wheal diameter of 3 mm or greater in the Nottingham Gedling cohort, and an increased specific IgE level (≥ 0.35 kU_A/L) or positive SPT wheal diameter of 3 mm or greater in the Southampton cohort.

TABLE II. Minor allele frequencies for key SNPs genotyped

SNP	Allele (major/minor)	British 1958 Birth Cohort (n = 3853)	ALSPAC (n = 1324)	Nottingham Gedling (n = 1225)	Southampton (n = 676)
rs7835920	A/C	0.25	0.27	0.25	0.25
rs7835153	A/G	0.42	0.42	0.43	0.43
rs11244450	G/A	0.09	0.08*	0.10	0.09
rs4936003	A/G	0.24	0.24*	0.23	0.26
rs6561505	G/A	0.08	0.08*	0.12	0.12
rs10514054	C/A	0.12	0.12	0.39	0.36
rs6082335	G/A	0.38	0.37*	0.25	0.25

Minor allele frequencies are shown for studied SNPs selected for prospective genotyping in all independent cohorts.

* r^2 Quality metrics for imputed SNPs with MACH 1.0 software are 0.86 (rs11244450), 0.96 (rs4936003), 0.97 (rs6561505), and 0.99 (rs6082335).

analysis was performed with STATA version 10.1 software (StataCorp, College Station, Tex) to assess SNP association with (Ln) serum total IgE levels.

All serum total IgE data were (Ln) transformed. Adjustments were taken for potential confounders: sex, date and time of collection, postal delay, laboratory batch, and nurse in the British 1958 Birth Cohort. Results presented are unadjusted in ALSPAC and the Nottingham Gedling and Southampton cohorts. SNP effect sizes for minor alleles were assessed by using odds ratios for dichotomized traits and β -trend calculations for continuous traits. Positive replication was defined by a P value of .05 or less for the same allele and the same direction of effect.

Combined analysis. Genotype data across all 4 cohorts were grouped by phenotype: increased specific IgE levels, positive SPT responses, and serum total IgE levels. Combined analyses for increased specific IgE levels and positive SPT responses were conducted by totaling allele counts of genotyped SNPs in all cases and control subjects to calculate joint P values and effect size. Total IgE results were combined for the Nottingham Gedling and Southampton cohorts only. Patterns of risk associations and effect sizes were calculated for each SNP minor allele.

RESULTS

Discovery GWAS

The results of the 1958 Birth Cohort analysis are summarized by the quantile-quantile plot shown in Fig 1. This shows a small number of SNPs with extreme P values and additional SNPs with more modest effect sizes influencing atopy susceptibility, as defined by increased specific IgE levels.

A single SNP (rs6561505) on chromosome 13q14 located within intron 12 of the Fibronectin type III domain containing protein (*FNDC3A*) gene reached genome-wide significance ($P = 2.15 \times 10^{-9}$). A further 4 SNPs were identified by using the lower threshold of a P value of 10^{-5} or less: rs7835153 ($P = 4.50 \times 10^{-7}$), rs7835920 ($P = 5.54 \times 10^{-7}$), rs17202769 ($P = 1.39 \times 10^{-6}$), and rs11244450 ($P = 6.50 \times 10^{-6}$) on chromosomes 8q22, 8q12 (2 SNPs), and 10q26, respectively. A further 11 SNPs were identified based on the predefined criteria for clustering with a P value of 10^{-4} or less (≥ 3 SNPs within 100 kb) on chromosomes 8q12 (2 SNPs), 11q24 (3 SNPs), 18q22 (3 SNPs), and 20p13 (3 SNPs).

Further analysis of these 16 SNPs revealed that they were located in 7 different chromosomal regions. The highest statistically significant SNPs in each region were rs7835920 (8q12 risk), rs7835153 (8q22 protective), rs11244450 (10q26 risk), rs4936003 (11q24 risk), rs6561505 (13q14 risk), rs10514054 (18q22 risk), and rs6082335 (20p13 risk; Table III).

In silico replication

All 7 loci identified were studied in ALSPAC. Only 3 of the key SNPs (highest statistical significance) were genotyped in ALSPAC (rs7835920, rs7835153, and rs10514054), of which none showed replicated association with SPT positivity. Imputed data

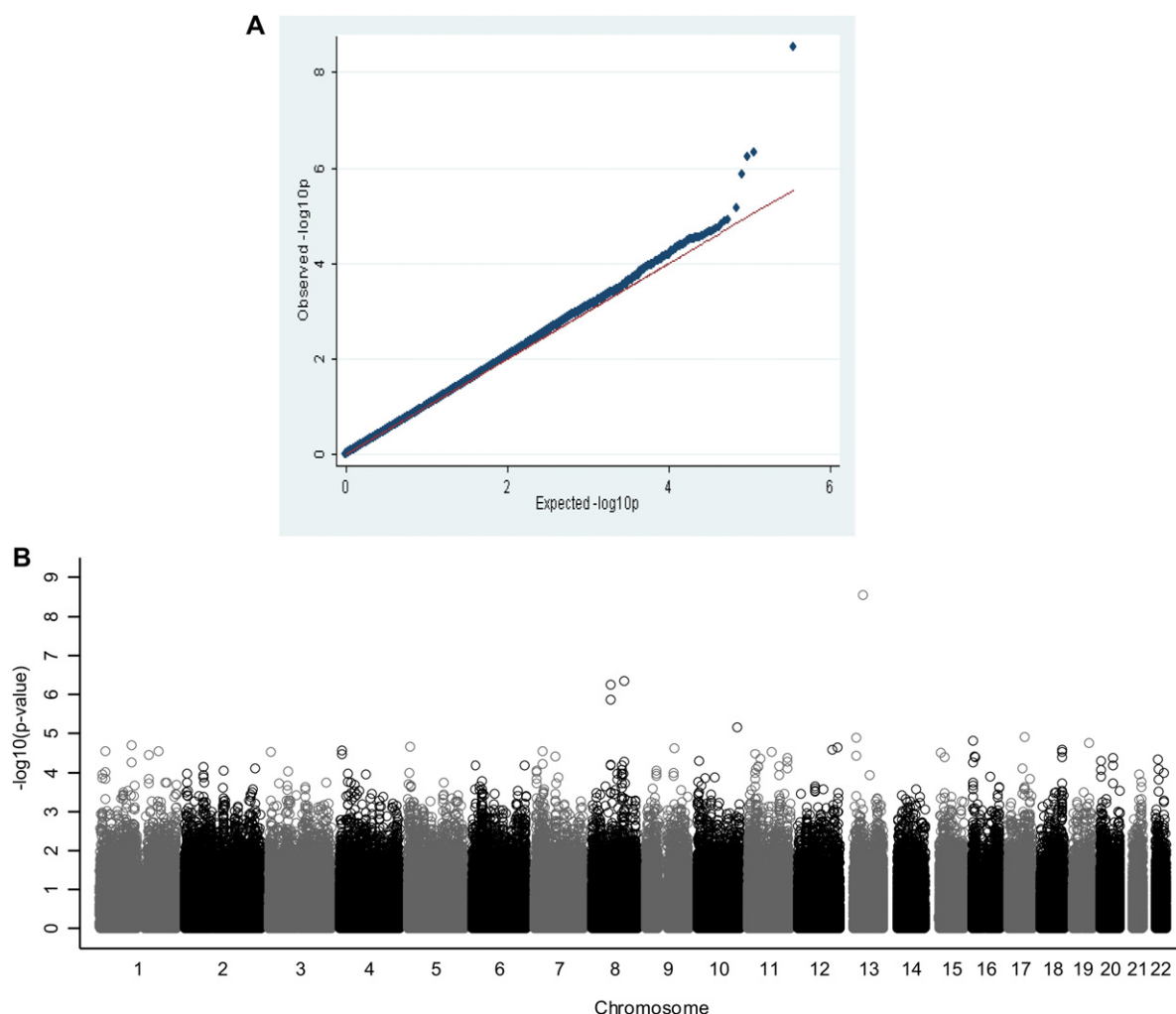


FIG 1. Results of the discovery GWAS. **A**, Quantile-quantile plot of observed versus expected log (base 10) P values for SNPs passing quality control measures in the British 1958 Birth Cohort (increased specific IgE levels). The straight line shows the distribution of SNPs under the null hypothesis. **B**, Manhattan plot of 459,334 autosomal SNPs with minor allele frequencies of greater than 5% and Hardy-Weinberg equilibrium P values of greater than .05 ordered by chromosome position for increased specific IgE levels.

were provided for the remaining 4 nongenotyped SNPs, and association was seen with rs4936003 ($P = 9.93 \times 10^{-4}$) and marginally with rs11244450 ($P = .045$; Table III).

Replication genotyping

All 7 key SNPs identified in the discovery cohort using the above criteria were genotyped in the Nottingham Gedling and Southampton cohorts. None of the SNPs showed evidence of association in the Nottingham Gedling or Southampton cohorts for either specific IgE levels or positive SPT responses.

Combined analyses

Combined analyses of count data for increased specific IgE levels and positive SPT responses across cohorts were conducted to estimate joint P values and effect sizes. The results of association in all cohorts and combined analyses are shown in Table III. Forest plots of the per-allele effect sizes for each of the SNPs are presented (Figs 2 and 3). Results for all SNPs are listed in Table E1 (available in this article's Online Repository at www.jacionline.org).

Pooled data showed significant associations with increased specific IgE levels for all 7 SNPs, although these effects were driven by the initial British 1958 Birth Cohort dataset. No SNPs showed significant association with positive SPT responses.

Total IgE analyses

Six studied SNPs also showed association with serum total IgE levels in the British 1958 Birth Cohort: rs7835920 ($P = 5.79 \times 10^{-4}$), rs7835153 ($P = 3.47 \times 10^{-5}$), rs11244450 ($P = 2.14 \times 10^{-4}$), rs6561505 ($P = 7.75 \times 10^{-5}$), rs10514054 ($P = 6.52 \times 10^{-3}$), and rs6082335 ($P = 4.37 \times 10^{-2}$). A single SNP, rs10514054, replicated association with serum total IgE levels in the Southampton cohort ($P = .03$) but showed opposing directions of effect for the minor allele (British 1958 Birth Cohort: β coefficient of 1.14 [95% CI, 1.04-1.26] and Southampton cohort: β coefficient of 0.53 [95% CI, 0.30-0.94]). Combined analysis of total IgE levels was carried out for the Nottingham Gedling and Southampton cohorts, and no SNPs met statistical significance.

DISCUSSION

We have conducted a large-scale population-based GWAS for increased specific IgE levels to common allergens in 3853 subjects from the British 1958 Birth Cohort, with follow-up investigations in 3225 subjects from 3 additional cohorts using atopy defined by increased specific IgE levels, positive SPT responses, or both. Overall, we did not identify replicated evidence for any of the atopy susceptibility loci identified in the discovery cohort by using increased specific IgE levels to define atopy.

The quantile-quantile plot for all SNPs in the British 1958 Birth Cohort-specific IgE dataset provides an indication of the likelihood of identifying susceptibility loci. Deviation of plotted results from the predicted distribution of *P* values in the absence of genetic effects is seen for a small number of SNPs, predominantly at the extreme *P* values. However, in addition, we saw a large number of SNPs with small effect sizes above the line, indicating there are probably numerous regions containing SNPs with small effects. This suggests that genetic influences on specific IgE levels do not come from a single or even a few major genes with large effect sizes but rather a larger number of loci with modest effects.

Seven loci were identified in the initial genome-wide association scan for susceptibility to atopy, all of which are in regions that have previously reported linkage to IgE levels and the development of various atopic traits: 8q12,²⁴ 8q22,^{25,26} 10q26,²⁷ 11q24,²⁸ 13q14,²⁹ 18q22,³⁰ and 20p13.^{31,32} Chromosome 13q14 is a region supported by both previous linkage and association results in which a number of studies have been conducted to examine potential candidate genes, including *PHF11* and *CYSLTR2*.^{33,34} The pivotal SNP in this locus reached genome-wide significance for increased risk of atopy and is located in *FNDC3A*, a gene not previously implicated in atopy or related traits.

To replicate our GWAS findings, we used a combination of *in silico* replication, prospective genotyping, and combined analyses. First, we attempted to replicate the SNP rs6561505, which met genome-wide significance for specific IgE levels. This SNP was not associated with atopy defined by increased specific IgE levels or positive SPT responses in the additional 3 cohorts. The minor allele for this SNP also increased the risk of increased serum total IgE levels in the British 1958 Birth Cohort but not in additional cohorts (*n* = 3225). Pooled results for increased specific IgE levels showed a significant combined effect of an OR of 1.49 (95% CI, 1.28-1.75) for the risk allele.

Although our study focused on the identification of signals reaching conventional GWAS significance, we also implemented a more inclusive region-based strategy and prospectively genotyped an additional 6 SNPs within loci showing at least some evidence of association. For the majority of SNPs genotyped, we saw no evidence for association in the additional cohorts: rs7835153 (8q22), rs11244450 (10q26), and rs10514054 (18q22). Despite significant associations for increased specific IgE levels in the British 1958 Birth Cohort, there is little evidence of consistent association in the additional cohorts for these SNPs. Overall, it seems unlikely that these 3 loci listed confer markedly increased susceptibility to atopy in these populations.

A single SNP, rs4936003 (11q24), showed replicated association with positive SPT responses in ALSPAC (*P* = 7.33×10^{-4}), with the same direction of effect for the minor allele (OR, 1.43; 95% CI, 1.16-1.76) as in the British 1958 Birth Cohort (OR, 1.27; 95% CI, 1.13-1.43). This is the only SNP that showed association with atopy across 2 independent cohorts by using subjects of

different ages and definitions of atopy. Because this SNP intronic of the *KIRREL3* gene did not show evidence of association with total IgE levels, this indicates that it might play a role in sensitization to allergens different from influencing IgE production *per se*. One study has shown the involvement of the gene product mKirre in the support of hematopoietic stem cells by stromal cells.³⁵

In addition, we identified some suggestive evidence for association for rs7835920 (8q12) and rs6082335 (20p13). For these SNPs, although not all replication results are statistically significant, a consistent effect can be seen for the risk allele throughout. The results for these 2 SNPs suggest that there might be genes underlying atopy susceptibility in these regions. Combined analyses for increased specific IgE levels showed effect sizes of ORs of 0.85 (*P* = 8.20×10^{-7}) and 1.22 (*P* = 2.78×10^{-5}), respectively, although these were driven by the initial signal in the British 1958 Birth Cohort.

Because 4 datasets were available with total IgE measures in addition to combined analysis on available raw data from the Gedling and Southampton cohorts only, we conducted a meta-analysis of the individual study per-allele effect estimates. Three SNPs met nominal significance (*P* ≤ .01), although as anticipated, these *P* values were driven by the British 1958 Birth Cohort, which formed the largest contribution. This analysis also confirmed high levels of between-study heterogeneity of effect sizes (see Table E2 in this article's Online Repository at www.jacionline.org).

We repeated the GWAS in the 1958 Birth Cohort using a more stringent threshold to define atopy (ie, specific IgE ≥ 3.5 kU/L), reducing the number of cases to 477. The association results of this sensitivity analysis did not identify any SNPs meeting genome-wide significance, and results for SNPs identified in the current article did not differ distinctly between the 2 sets of analyses (results not shown). We also examined potential association for SNPs in candidate genes previously identified in a smaller dataset of the British 1958 Birth Cohort.³⁶ In the British 1958 Birth cohort, association with increased specific IgE levels was seen with SNPs within previously identified atopy genes, including *FCERIA* (5 SNPs, lowest *P* = .002), interleukin 13 (2 SNPs, lowest *P* = .02), and *STAT6* (6 SNPs, lowest *P* = .003).^{34,37,38} Although some evidence of association was seen for both specific and total IgE levels in our current study, none of these signals met conventional GWAS significance criteria in the larger dataset. We were unable to formally examine association of genes around the HLA locus because of limitations in SNP coverage on the Illumina 550K Array.

A number of factors might have contributed to difficulty in replication. First, "winner's curse bias" might have resulted in inflated effect size estimates obtained from the discovery cohort, requiring much larger replication studies to have sufficient power to detect unbiased effect sizes. Numbers of study subjects used in this study might have been unlikely to yield enough statistical power to detect variants with modest effects, particularly in the presence of substantial allelic heterogeneity.³⁹ Our power estimations calculate that by using current sample sizes and an average minor allele frequency of 24% (SD 0.12) in pivotal SNPs, more than 16,000 subjects are required in replication cohorts to have 80% power to detect risk allele ORs of greater than 1.2.

Second, heterogeneity across study populations in both phenotype definitions and variable coverage of SNPs across the genome in genotyping arrays might have complicated analyses. Not all of the key SNPs studied were genotyped in ALSPAC, requiring the use of imputed data. Although the *r*² quality metrics

TABLE III. Association with atopic phenotypes for studied SNPs: Increased specific IgE level, positive SPT response, and Ln(total IgE)

Chromosome	SNP (minor allele), gene	Increased specific IgE level		Positive SPT response		
		B58C	Southampton	ALSPAC	Nottingham Gedling	Southampton
8q12	rs7835920 (C), intergenic	5.54×10^{-7}*	.30	.35	.06*	.49
8q22	rs7835153 (G), <i>ANKRD46</i>	4.50×10^{-7}†	.17	.13	.82	.17
10q26	rs11244450 (A), intergenic	6.50×10^{-6}*	.33	.045†	.73	.12
11q24	rs4936003 (G), <i>KIRREL3</i>	4.20×10^{-5}*	.33	7.33×10^{-4}*	.32	.69
13q14	rs6561505 (A), <i>FNDC3A</i>	2.15×10^{-9}*	.44	.73	.16	.27
18q22	rs10514054 (A), <i>NETO1</i>	2.54×10^{-5}*	.17	.06*	.38	.60
20p13	rs6082335 (A), intergenic	5.00×10^{-5}*	.10	.80	.97	.31

Results are shown for studied SNPs identified in the discovery GWAS cohort: the British 1958 Birth Cohort (B58C) and the replication cohorts ALSPAC, Nottingham Gedling, and Southampton. Combined *P* values and effect sizes have been calculated by using available raw data. Combined *P* values of .05 or less are shown in boldface. *Risk minor allele. †Protective minor allele.

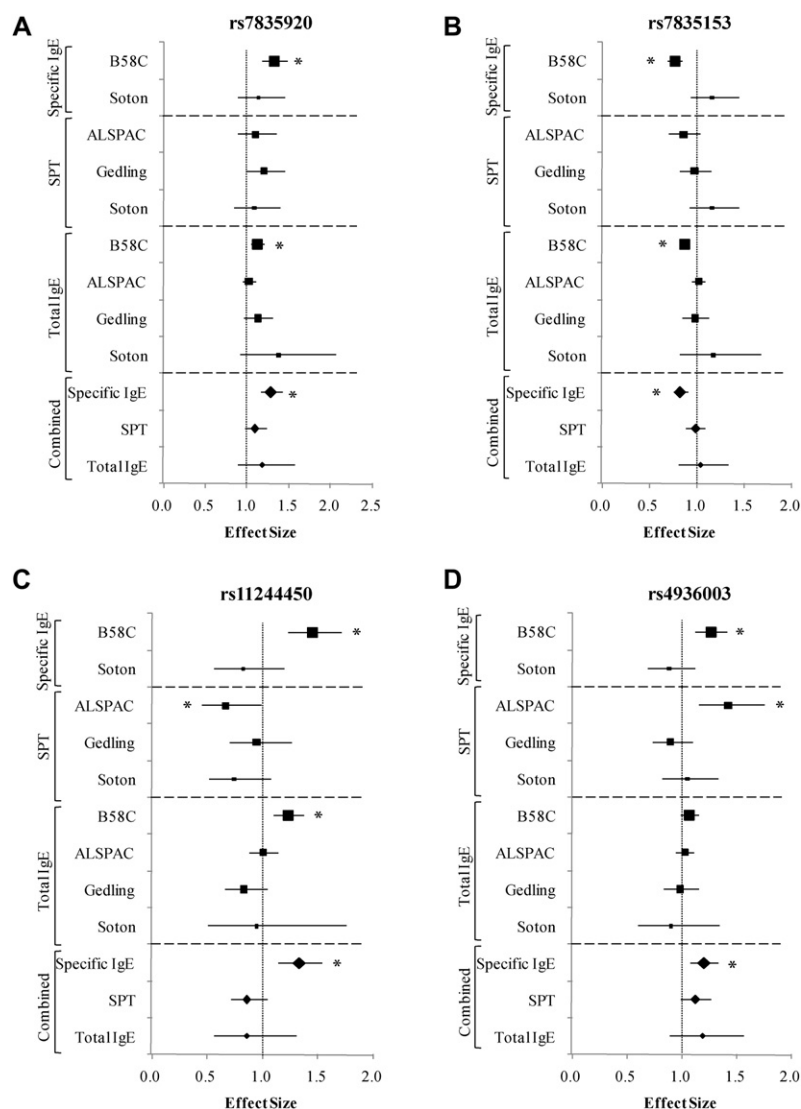


FIG 2. Forest plots of studied SNPs 1 to 4: rs7835920 (8q12; **A**), rs7835153 (8q22; **B**), rs11244450 (10q26; **C**), and rs4936003 (11q24; **D**). Per-allele summary effect sizes for the minor allele are shown for atopic phenotypes: increased specific IgE level (OR), positive SPT response (OR), and serum total IgE level (β coefficient). Each row represents 1 study, relative sizes of data points are proportional to the cohort sample size, and horizontal lines represent 95% CIs. Diamonds represent the meta-analyses. *Statistical significance ($P \leq .05$).

TABLE III. (Continued).

Ln (total IgE)				Combined		
B58C	ALSPAC	Nottingham Gedling	Southampton	Elevated specific IgE	Positive SPT	Ln(total IgE)
$5.79 \times 10^{-4*}$	.55	.13	.12	$8.20 \times 10^{-7*}$	.11	.24
$3.47 \times 10^{-5\dagger}$	.45	.82	.38	$7.00 \times 10^{-5\dagger}$	.82	.72
$2.14 \times 10^{-4*}$	.89	.13	.87	$2.08 \times 10^{-4*}$	.14	.49
.054 [†]	.48	.86	.63	$4.36 \times 10^{-4*}$	.052*	.23
$7.75 \times 10^{-5*}$	.87	.84	.86	$5.45 \times 10^{-7*}$	.31	.34
$6.52 \times 10^{-3*}$	.13	.19	.03 [†]	$1.32 \times 10^{-3*}$	.90	.48
.04*	.96	.34	.43	$2.78 \times 10^{-5*}$	.71	.83

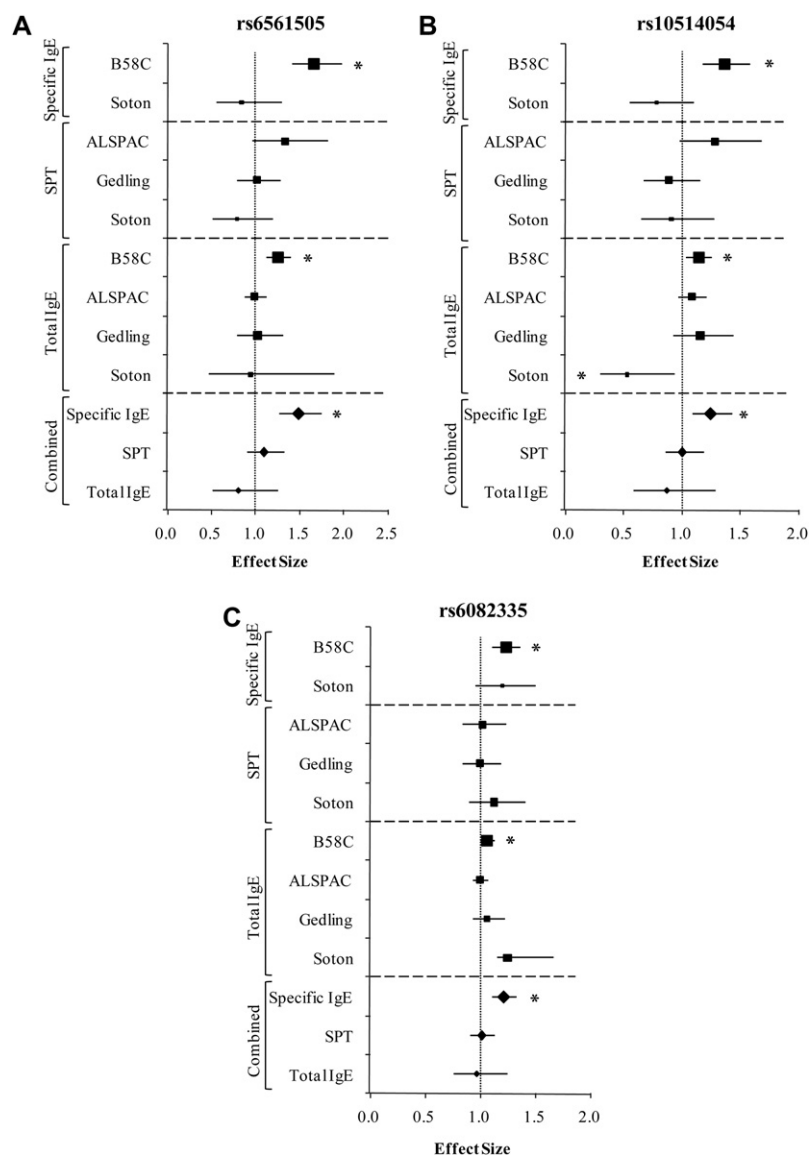


FIG 3. Forest plots of studied SNPs 5 to 7: rs6561505 (13q14; **A**), rs10514054 (18q22; **B**), and rs6082335 (20p13; **C**). Per-allele summary effect sizes for the minor allele are shown for atopic phenotypes: increased specific IgE level (OR), positive SPT response (OR), and serum total IgE level (β coefficient). Each row represents 1 study, relative sizes of data points are proportional to the cohort sample size, and horizontal lines represent 95% CIs. Diamonds represent the meta-analyses. *Statistical significance ($P \leq .05$).

for these SNPs were relatively high, this was a further limitation during replication.

Many SNPs showing association with increased specific IgE levels in the discovery GWAS were also associated with serum total IgE levels in the 1958 birth cohort. In addition, measured increased specific IgE levels and positive SPT responses for the same subjects in the Southampton cohort correlated well (Pearson $r^2 = 0.73$, $P < .001$). However, the relationship of these phenotypes is unclear, and it is likely that the use of different atopy markers across cohorts might have contributed to difficulty in replication because of different genetic contributions to these related phenotypes. Despite some overlap in SNP association, loci identified for atopy by specific IgE level and positive SPT response in this study appear to be unique compared with the studies that have used increased total serum IgE levels, suggesting that the processes controlling sensitization might differ from those controlling the level of IgE production.

Although previous research with ALSPAC suggests that an allergen panel consisting of only 3 aeroallergens is representative, there is still some risk of phenotype misclassification in which atopic subjects will be considered as control subjects in the absence of a more extensive panel.¹⁵ In addition, variations in the antigen panels used across cohorts might have complicated replication, as well as differences in population demographics. The British 1958 Birth Cohort, the Nottingham Gedling cohort, and the Southampton cohort are adult populations, whereas ALSPAC had a mean age of 7.5 years at the time of atopy data collection.

The main conclusion that can be drawn from this study is that overall the evidence we have provided points to a large number of genetic factors influencing atopy risk, each with a small contribution. Therefore the detection of further susceptibility loci will require GWASs with more complete genome SNP coverage and using larger sample sizes together with the combination of results across multiple studies. Our results have significant implications for the design of reliable studies to identify genes regulating increased specific IgE levels and contributing to atopy. This study has highlighted the importance for well-designed and large GWASs to address these issues.

We thank all members of the study cohorts taking part in this study, particularly those who provided consent to use of their DNA for genetic epidemiologic analyses, and the research staff who contributed to the successful completion of all field studies.

Key Messages

- Using the British 1958 Birth Cohort (n = 3853), we have conducted the first GWAS with increased specific IgE levels as a marker of atopy and identified 7 potential susceptibility loci.
- Loci identified showed limited evidence for replication in 3 independent white cohorts (n = 3225), potentially inferring phenotype and genotype heterogeneity in atopy defined by increased specific IgE levels, positive SPT responses, or both.

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TABLE E1. Association results between studied SNPs detailing per-allele *P* values and effect sizes measured by using ORs for dichotomized traits: Increased specific IgE levels and positive SPT responses (β -trend coefficient for continuous trait: serum total IgE level)**(i) Chromosome 8 (59,471,286 bp)**

SNP	Phenotype	Cohort	No.		Effect (95% CI)			<i>P</i> value
			Cases	Control subjects	I95	Size	u95	
rs7835920 A/C	Increased specific IgE level	B58C	1083	2770	1.188	1.328	1.485	5.54×10^{-7}
		Southampton	292	376	0.889	1.140	1.461	3.02×10^{-1}
	Positive SPT response	ALSPAC	281	1042	0.898	1.104	1.356	3.49×10^{-1}
		Gedling	385	822	0.992	1.206	1.465	5.98×10^{-2}
		Southampton	377	291	0.850	1.091	1.401	4.95×10^{-1}
	Serum total IgE level	B58C	3873		1.055	1.133	1.217	5.79×10^{-4}
		ALSPAC	1423		0.947	1.024	1.107	5.52×10^{-1}
		Gedling	1117		0.965	1.129	1.322	1.29×10^{-1}
		Southampton	663		0.920	1.380	2.069	1.19×10^{-1}
	Combined	Specific IgE level	1375	3146	1.165	1.289	1.426	8.20×10^{-7}
		Positive SPT response	1043	2155	0.977	1.100	1.238	1.14×10^{-1}
		Total IgE level	1780		0.895	1.185	1.570	2.36×10^{-1}

(ii) Chromosome 8 (101,642,996 bp), *ANKRD46*

SNP	Phenotype	Cohort	No.		Effect (95% CI)			<i>P</i> value
			Cases	Control subjects	I95	Size	u95	
rs7835153 A/G	Increased specific IgE level	B58C	1082	2759	0.695	0.770	0.852	4.50×10^{-7}
		Southampton	290	380	0.936	1.165	1.449	1.71×10^{-1}
	Positive SPT response	ALSPAC	282	1036	0.715	0.864	1.045	1.32×10^{-1}
		Gedling	390	828	0.825	0.980	1.164	8.19×10^{-1}
		Southampton	377	293	0.936	1.165	1.450	1.72×10^{-1}
	Serum total IgE level	B58C	3873		0.823	0.876	0.933	3.47×10^{-5}
		ALSPAC	1423		0.958	1.027	1.102	4.51×10^{-1}
		Gedling	1129		0.856	0.984	1.132	8.23×10^{-1}
		Southampton	665		0.821	1.176	1.686	3.76×10^{-1}
	Combined	Specific IgE level	1372	3139	0.758	0.831	0.910	7.00×10^{-5}
		Positive SPT response	1049	2157	0.889	0.988	1.098	8.17×10^{-1}
		Total IgE level	1794		0.816	1.047	1.343	7.18×10^{-1}

(iii) Chromosome 10 (126,026,768 bp)

SNP	Phenotype	Cohort	No.		Effect (95% CI)			<i>P</i> value
			Cases	Control subjects	I95	Size	u95	
rs11244450 G/A	Increased specific IgE level	B58C	1079	2763	1.237	1.460	1.722	6.50×10^{-6}
		Southampton	291	374	0.571	0.831	1.209	3.32×10^{-1}
	Positive SPT response	ALSPAC	242	1042	0.453	0.672	0.998	4.47×10^{-2}
		Gedling	382	826	0.710	0.950	1.272	7.33×10^{-1}
		Southampton	376	289	0.518	0.747	1.079	1.20×10^{-1}
	Serum total IgE level	B58C	3873		1.103	1.231	1.374	2.14×10^{-4}
		ALSPAC	1423		0.885	1.009	1.151	8.89×10^{-1}
		Gedling	1120		0.666	0.838	1.054	1.31×10^{-1}
		Southampton	660		0.512	0.950	1.766	8.72×10^{-1}
	Combined	Specific IgE level	1370	3137	1.144	1.331	1.547	2.08×10^{-4}
		Positive SPT response	1040	2157	0.718	0.868	1.049	1.42×10^{-1}
		Total IgE level	1780		0.569	0.863	1.308	4.86×10^{-1}

(iv) Chromosome 11 (126,258,841 bp), *KIRREL3*

SNP	Phenotype	Cohort	No.		Effect (95% CI)			<i>P</i> value
			Cases	Control subjects	I95	Size	u95	
rs4936003 A/G	Increased specific IgE level	B58C	1073	2754	1.133	1.270	1.425	4.20×10^{-5}
		Southampton	295	381	0.694	0.886	1.132	3.32×10^{-1}
	Positive SPT response	ALSPAC	282	1042	1.161	1.429	1.759	7.33×10^{-4}
		Gedling	386	826	0.735	0.902	1.106	3.21×10^{-1}
		Southampton	382	294	0.824	1.052	1.343	6.85×10^{-1}

(Continued)

TABLE E1. (Continued)

(iv) Chromosome 11 (126,258,841 bp), *KIRREL3*

SNP	Phenotype	Cohort	No.		Effect (95% CI)			P value
			Cases	Control subjects	195	Size	u95	
	Serum total IgE level	B58C	3873		0.999	1.075	1.156	5.42×10^{-2}
		ALSPAC	1423		0.949	1.030	1.119	4.77×10^{-1}
		Gedling	1125		0.840	0.986	1.158	8.64×10^{-1}
		Southampton	671		0.609	0.906	1.350	6.28×10^{-1}
Combined		Specific IgE level	1368	3135	1.085	1.204	1.335	4.36×10^{-4}
		Positive SPT response	1050	2162	0.999	1.127	1.271	5.17×10^{-2}
		Total IgE level	1769		0.897	1.189	1.577	2.29×10^{-1}

(v) Chromosome 13 (48,651,730 bp), *FNDC3A*

SNP	Phenotype	Cohort	No.		Effect (95% CI)			P value
			Cases	Control subjects	195	Size	u95	
rs6561505 G/A	Increased specific IgE level	B58C	1081	2768	1.410	1.671	1.979	2.15×10^{-9}
		Southampton	288	370	0.553	0.846	1.296	4.42×10^{-1}
Positive SPT response		ALSPAC	242	1042	0.973	1.333	1.826	7.76×10^{-2}
		Gedling	394	825	0.920	1.238	1.665	1.58×10^{-1}
		Southampton	371	286	0.520	0.791	1.204	2.73×10^{-1}
Serum total IgE level		B58C	3873		1.123	1.258	1.409	7.75×10^{-5}
		ALSPAC	1423		0.875	0.990	1.120	8.70×10^{-1}
		Gedling	1167		0.799	1.027	1.321	8.35×10^{-1}
		Southampton	675		0.466	0.940	1.896	8.63×10^{-1}
Combined		Specific IgE level	1369	3138	1.275	1.492	1.746	5.45×10^{-7}
		Positive SPT response	1007	2153	0.914	1.103	1.331	3.05×10^{-1}
		Total IgE level	1842		0.517	0.807	1.259	3.44×10^{-1}

(vi) Chromosome 18 (68,532,581 bp)

SNP	Phenotype	Cohort	No.		Effect (95% CI)			P value
			Cases	Control subjects	195	Size	u95	
rs10514054 C/A	Increased specific IgE level	B58C	1082	2770	1.183	1.371	1.589	2.54×10^{-5}
		Southampton	290	377	0.557	0.786	1.109	1.69×10^{-1}
Positive SPT response		ALSPAC	282	1041	0.980	1.287	1.690	6.92×10^{-2}
		Gedling	385	831	0.678	0.887	1.160	3.81×10^{-1}
		Southampton	377	290	0.652	0.913	1.280	5.99×10^{-1}
Serum total IgE level		B58C	3873		1.038	1.143	1.259	6.52×10^{-3}
		ALSPAC	1423		0.976	1.087	1.210	1.28×10^{-1}
		Gedling	1126		0.929	1.158	1.443	1.90×10^{-1}
		Southampton	662		0.303	0.534	0.943	3.03×10^{-2}
Combined		Specific IgE level	1372	3147	1.090	1.248	1.429	1.32×10^{-3}
		Positive SPT response	1044	2162	0.860	1.010	1.187	9.01×10^{-1}
		Total IgE level	1782		0.587	0.869	1.288	4.84×10^{-1}

(vii) Chromosome 20 (2,114,427 bp)

SNP	Phenotype	Cohort	No.		Effect (95% CI)			P value
			Cases	Control subjects	195	Size	u95	
rs6082335 G/A	Increased specific IgE level	B58C	1076	2741	1.115	1.235	1.367	5.00×10^{-5}
		Southampton	290	376	0.962	1.215	1.509	1.04×10^{-1}
Positive SPT response		ALSPAC	242	1042	0.847	1.026	1.242	7.95×10^{-1}
		Gedling	385	826	0.841	1.003	1.196	9.73×10^{-1}
		Southampton	375	291	0.897	1.124	1.410	3.09×10^{-1}
Serum total IgE level		B58C	3873		1.002	1.068	1.138	4.37×10^{-2}
		ALSPAC	1423		0.934	1.002	1.075	9.61×10^{-1}
		Gedling	1123		0.931	1.069	1.227	3.44×10^{-1}
		Southampton	662		1.158	1.248	1.674	4.34×10^{-1}
Combined		Specific IgE level	1366	3117	1.110	1.218	1.338	2.78×10^{-5}
		Positive SPT response	1003	2159	0.916	1.020	1.136	7.14×10^{-1}
		Total IgE level	1785		0.757	0.973	1.249	8.28×10^{-1}

Data for serum total IgE level are corrected for sex and sample collection differences at age 44 to 45 years in the British 1958 Birth Cohort (B58C) and uncorrected in the ALSPAC and Nottingham Gedling and Southampton cohorts. Effect sizes are presented for the minor (second) allele.

195, Lower 95th percentile; u95, upper 95th percentile.

TABLE E2. Meta-analysis of individual per-allele study effect sizes of studied SNPs for serum total IgE levels weighted inversely proportionally to study variance

SNP	Study heterogeneity (<i>P</i> value)	Effect (95% CI)			<i>P</i> value
		l95	Size	u95	
rs7835920 G/A	.166	−1.146	1.091	1.038	6.67×10^{-4}
rs7835153 C/A	.005	−0.990	0.948	0.907	.017
rs11244450 G/A	.010	−1.181	1.092	1.009	.028
rs4936003 A/G	.638	−1.100	1.045	0.993	.092
rs6561505 G/A	.036	−1.206	1.114	1.030	.007
rs10514054 C/A	.071	−1.187	1.110	1.037	.003
rs6082335 G/A	.144	−1.098	1.052	1.007	.023

A meta-analysis of individual per-allele effect sizes across all studies for total IgE levels was carried out by using STATA 10.1 software with weights inversely proportionate to study variance. Effect sizes are presented for the minor (second) allele.