



Human leukocyte antigen class II variants and adult-onset asthma: does occupational allergen exposure play a role?

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ABSTRACT Recently, a locus centred on rs9273349 in the *HLA-DQ* region emerged from genome-wide association studies of adult-onset asthma. We aimed to further investigate the role of human leukocyte antigen (HLA) class II in adult-onset asthma and a possible interaction with occupational exposures.

We imputed classical HLA-II alleles from 7579 single-nucleotide polymorphisms in 6025 subjects (1202 with adult-onset asthma) from European cohorts: ECRHS, SAPALDIA, EGEA and B58C, and from surveys of bakers and agricultural workers. Based on an asthma-specific job–exposure matrix, 2629 subjects had ever been exposed to high molecular weight (HMW) allergens. We explored associations between 23 common HLA-II alleles and adult-onset asthma, and tested for gene–environment interaction with occupational exposure to HMW allergens. Interaction was also tested for rs9273349.

Marginal associations of classical HLA-II alleles and adult-onset asthma were not statistically significant. Interaction was detected between the *DPB1*03:01* allele and exposure to HMW allergens ($p=0.009$), in particular to latex ($p=0.01$). In the unexposed group, the *DPB1*03:01* allele was associated with adult-onset asthma (OR 0.67, 95%CI 0.53–0.86). HMW allergen exposures did not modify the association of rs9273349 with adult-onset asthma.

Common classical HLA-II alleles were not marginally associated with adult-onset asthma. The association of latex exposure and adult-onset asthma may be modified by *DPB1*03:01*.



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In an analysis involving >6000 European adults, classical HLA-II alleles were not associated with adult-onset asthma <http://ow.ly/xTdFm>

Support statement: This gene–environment interaction analysis was supported by IAGO ANR-2010-PRSP-003, and by the Netherlands Organisation for Scientific Research (NWO)/French Ministry of Foreign and European affairs Van Gogh programme for French–Dutch cooperation. Genotyping of the ESE and industrial cohorts, and part of B58C was funded by the GABRIEL asthma genetic consortium supported by a contract from the European Commission (018996) and grants from the French Ministry of Research, the Wellcome Trust (WT084703MA), Asthma UK and The COLT Foundation. For B58C, Asthma UK provided funding for the asthma-specific job–exposure matrix coding of occupational exposures and investigation of their associations with asthma, and the COLT Foundation provided funding for extension of this work to look at other allergic outcomes. Further sources of funding for the participating cohorts are given in the online supplementary material. None of the funders had any role in study design, data collection and analysis, or preparation of the manuscript.

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Introduction

The highly polymorphic human leukocyte antigen (HLA) class II molecules (DR, DQ and DP) are expressed on the surface of antigen-presenting cells, where they present peptides of exogenous origin to T-cell receptors on CD4⁺ cells. Because of the pivotal role of HLA-II molecules in the recognition of allergen-derived peptides, HLA alleles have been extensively studied in hypothesis-driven candidate gene association studies for allergic sensitisation and asthma [1, 2]. HLA is also the most evaluated genetic risk factor for occupational asthma. Although results were inconsistent, a role of the *HLA-DQB1* locus in occupational asthma aetiology is plausible [3].

Evidence from several independent genome-wide association studies (GWAS) lend further support to the involvement of HLA-II loci in asthma, allergic sensitisation and increased total IgE level [4–11]. The GABRIEL consortium carried out the largest asthma GWAS to date, by genotyping 10 365 asthma patients and 16 110 controls recruited from 23 studies [4]. A locus centred on rs9273349 in the *HLA-DQ* region showed strong evidence of association in the overall sample ($p=7 \times 10^{-14}$; OR for C allele 1.18). This was the only single-nucleotide polymorphism (SNP) with genome-wide significance for asthma with onset at 16 years of age or older ($p=4 \times 10^{-8}$; OR 1.26). Interestingly, a more pronounced effect of rs9273349 was observed in later-onset asthma than in childhood-onset asthma, although the difference was not significant. A recent GWAS replicated the association of this SNP with asthma and also reported notably stronger associations in the adult compared with the childhood population [6].

It has been suggested that investigating gene–environment interactions may help to understand associations of HLA with asthma that were identified by GWAS [12]. Indeed, the strength of the association of HLA variants with asthma could depend on concomitant environmental exposures. Considering individuals' age of asthma onset can be instrumental in revealing gene–environment interactions [13, 14]. The age-dependent effects that were observed in GWAS may provide clues to the role of HLA, because late-onset asthma may have different underlying environmental risk factors than childhood asthma. In adults, occupational exposures are an important cause of incident asthma, with a population attributable risk of around 15% [15–17]. Investigating the effect of gene–environment interactions between HLA alleles and occupational exposures on asthma risk might reveal mechanisms mediated by specific HLA alleles and triggered by occupational sensitisers such as latex, flour or animal proteins.

The aim of the present study is to further investigate the role of HLA-II in adult-onset asthma in a meta-analysis of population-based and workforce-based studies. We imputed classical HLA-II alleles from 7579 SNPs, allowing an evaluation of association between HLA-II alleles and adult-onset asthma. In addition, we explored whether occupational exposure to high molecular weight (HMW) allergens modifies the effect of imputed HLA-II alleles and the rs9273349 SNP in the *HLA-DQ* region that emerged from the GABRIEL study on adult-onset asthma.

Methods

Population

We studied all European cohorts that were included in GABRIEL with detailed data available on occupational history: EGEA [18, 19], SAPALDIA [20, 21] and ECRHS [15, 22] (referred to as the “ESE cohorts”) [23], the British 1958 birth cohort (B58C) [16, 24], and industrial cohorts of Dutch and Danish agricultural workers (AGRI) [25], and Dutch bakery workers (BAKERY) [26]. We included nested asthma case–control samples (SAPALDIA, ECRHS, AGRI and BAKERY) as previously described [4], and all subjects from EGEA and B58C with data available on genome-wide genotyping, occupational history and adult-onset asthma. Adult-onset asthma was defined as ever having had asthma with an onset ≥ 16 years [4]. Subjects who had never had asthma were used as the reference group. Ethical approval was obtained for each cohort from the appropriate institutional ethics committee. Detailed cohort descriptions are given in the online supplementary material.

Genotyping

Genome-wide genotyping was conducted using the Human610 quad array panel (Illumina, San Diego, CA, USA) [4]. More details on genotyping of B58C are given in the online supplementary material. We investigated whether the association of the GABRIEL main signal (rs9273349) with adult-onset asthma was modified by occupational exposure. For completeness regarding GABRIEL HLA-II results, we further selected

This article has supplementary material available from www.erj.ersjournals.com

Received: April 09 2014 | Accepted: June 09 2014

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a subset of 18 tag SNPs using Haploview (Broad Institute, Cambridge, MA, USA) [27], and tested for gene–environment interaction with occupational exposure to HMW allergens (online supplementary material).

Imputation of classical HLA-II alleles

For all cohorts in the present analysis, we used 7579 SNPs of the extended major histocompatibility complex (MHC) (20–40 Mbp on chromosome 6) to impute classical HLA types for class II loci at four-digit resolution. Imputations were performed using BEAGLE software (University of Washington, Seattle, WA, USA) on the basis of haplotype patterns in 2767 unrelated individuals of European descent (this reference panel includes B58C subjects), as described previously [28]. Concordance between imputed data and genotyping (Dynal RELI SSO assays; Invitrogen, Carlsbad, CA, USA) [29] was assessed for *DRB1* and *DQB1* alleles in the 2310 B58C subjects included in the present paper who were not part of the reference panel for the BEAGLE imputations (online supplementary table E1). Imputation quality was generally high for common alleles ($r^2 > 0.8$). Pairwise linkage disequilibrium measures (D' and r^2) between classical HLA-II alleles and rs9273349 and the other 18 SNPs were estimated using Haploview (tables E2 and E3) [27].

Occupational exposure to HMW allergens

Occupational exposure assessment is described in detail in the online supplementary material. Briefly, in all cohorts, occupations were linked to an asthma-specific job–exposure matrix with a local expert re-evaluation step [19]. We only considered seven HMW allergens high-risk substances (namely animal proteins, fish or shellfish, mites, latex, flour, other plant antigens, and enzymes) that may cause asthma through a similar, predominantly IgE-associated mechanism. Subjects who were ever exposed to any of the seven HMW allergens were classified as “exposed”. The “unexposed” reference group consisted of subjects who were never exposed to any of the high-risk or low-risk jobs.

Statistical analysis

The ESE cohorts were pooled to increase statistical power and to avoid convergence problems, as was done before with EGEA and ECRHS data [30]. Raw data from B58C were not available for pooled analysis. We tested association of 23 common classical HLA-II alleles with allele frequency $> 5\%$ in each population with adult-onset asthma, assuming an additive genetic model. In ESE cohorts, population admixture was taken into account by including principal components for within-Europe diversity as covariates in the association analysis. Genetic effects were estimated in each cohort separately, stratified by exposure status. The overall (exposure-adjusted) gene effect was estimated by standard fixed-effects meta-analysis of ESE exposed, ESE unexposed, B58C exposed and B58C unexposed subjects. To evaluate gene–environment interaction, results in exposed and unexposed subjects from ESE and B58C were combined through fixed-effects meta-analysis to obtain odds ratios in the exposed and unexposed groups. Both the standard 1 degree of freedom (df) test of gene–environment interaction and the 2 df joint test of marginal genetic effects and gene–environment interaction were performed, the latter being generally considered to have greater power than the marginal test when the genetic effect is only expressed in exposed individuals [31, 32]. Additional analyses were performed by including the industrial cohorts in the meta-analysis of HMW allergen exposed workers, and performing the joint test and test for interaction. The industrial cohorts were not included to evaluate the marginal genetic effect because all subjects in these cohorts were exposed, so the interaction effect cannot be assumed to be independent of the exposed-only odds ratio. We did not adjust for multiple testing because of the prior evidence of HLA-II involvement in several GWAS. Instead, we used a p-value threshold of < 0.01 for the test of marginal association, joint test and test for interaction. More details are given in the online supplementary material.

Results

Descriptive characteristics of the HMW allergen exposed and unexposed study subjects are summarised in table 1. Subjects with childhood-onset asthma ($n = 685$) or missing age of onset ($n = 59$) were excluded from data analysis, leaving data from 6025 subjects for analysis. Latex was the predominant occupational exposure among subjects from the general population cohorts who were classified as HMW allergen exposed (from 64% exposed in B58C to 84% in SAPALDIA). Occupational exposure to HMW allergens was positively associated with adult-onset asthma in each of the four general population cohorts, with a fixed-effects summary odds ratio of 1.33 (95% CI 1.11–1.60) (fig. 1).

Table 2 shows results from association tests of 23 imputed classical HLA-II alleles, rs9273349 C/T, and adult-onset asthma in the ESE and B58C cohorts. Marginal associations of HLA-II alleles were not statistically significant ($p > 0.01$) but rs9273349 was, as expected, significantly associated with adult-onset asthma (OR for T allele 0.82, $p = 0.002$).

TABLE 1 Descriptive characteristics of the study cohorts by exposure status

	EGEA	SAPALDIA	ECRHS	B58C	AGRI	BAKERY
HMW allergen exposed						
Subjects n	192	121	214	1337	683	263
Age years	43.4±14.0	48.6±10.0	41.5±7.1	45.2±0.4	28.6±12.6	39.9±10.8
Males %	29	12	14	33	83	95
Asthma n	85	53	82	216	256	136
Adult-onset asthma	35 (41)	37 (70)	59 (72)	124 (57)	NA [#]	NA [#]
No asthma n	107	68	132	1121	427	127
HMW allergen exposures						
Latex	129 (67)	102 (84)	161 (75)	853 (64)	0 (0)	0 (0)
Animal proteins	33 (17)	1 (0.8)	15 (7.0)	198 (15)	475 (70)	0 (0)
Fish or shellfish	1 (0.5)	0 (0.0)	1 (0.4)	19 (1.4)	0 (0)	0 (0)
Flour	13 (6.8)	7 (5.7)	8 (3.7)	82 (6.1)	57 (8.3)	263 (100)
Other plant antigens	5 (2.6)	3 (2.4)	4 (1.9)	169 (13)	143 (21)	0 (0)
Mite and insect antigens	20 (10)	10 (8.3)	30 (14)	183 (14)	66 (9.7)	0 (0)
Enzymes	9 (4.7)	5 (4.1)	10 (4.7)	66 (4.9)	0 (0)	263 (100)
HMW allergen unexposed						
Subjects n	724	669	1202	1364		
Age years	42.0±15.1	53.7±10.7	43.5±7.1	45.2±0.4		
Males %	39	48	42	36		
Asthma n	294	276	347	201		
Adult-onset asthma	94 (32)	162 (59)	186 (54)	113 (56)		
No asthma	430	393	855	1163		

Data are presented as mean ± SD or n (%), unless otherwise stated. HMW: high molecular weight; NA: not available. [#]: age of asthma onset is not available in the industrial cohorts.

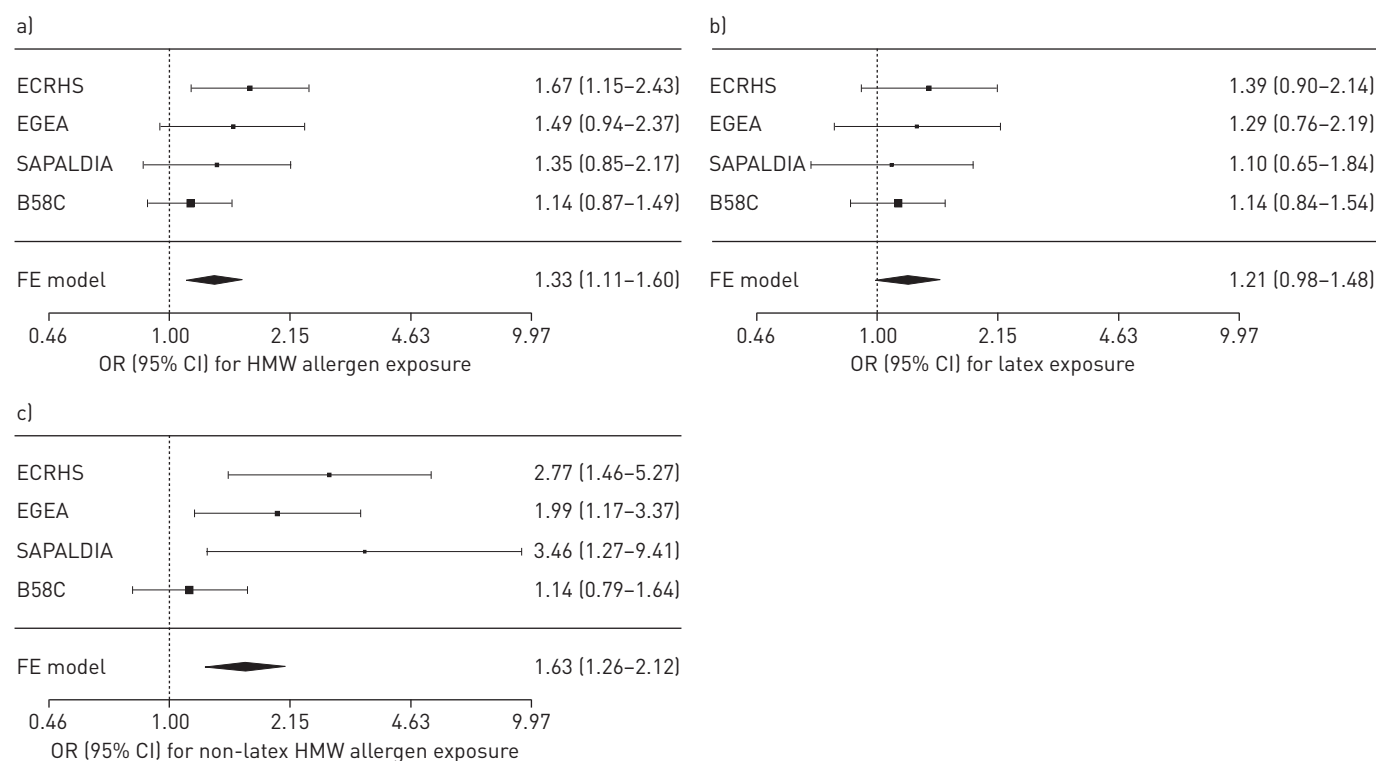


FIGURE 1 Forest plot of odds ratios and 95% confidence intervals for the association of occupational exposure to a) high molecular weight (HMW) allergens, b) latex and c) HMW allergens other than latex with adult-onset asthma in ECRHS, EGEA, SAPALDIA and B58C. The summary odds ratio was calculated using a fixed-effects meta-analysis (FE model).

TABLE 2 Association of imputed classical human leukocyte antigen (HLA) class II alleles, rs9273349, and adult-onset asthma in all ESE and B58C subjects, and according to ever having been exposed to occupational high molecular weight (HMW) allergens

HLA-II allele	AF [#]	All subjects [†]	HMW allergen exposed ⁺	HMW allergen unexposed [§]	p-value	
					Joint test	Interaction
<i>DRB1*01:01</i>	9.4	0.83 [0.68–1.01]	0.83 [0.57–1.21]	0.83 [0.66–1.04]	0.18	0.99
<i>DRB1*03:01</i>	12.6	1.09 [0.92–1.29]	1.05 [0.79–1.40]	1.11 [0.91–1.36]	0.52	0.75
<i>DRB1*04:01</i>	9.9	1.10 [0.91–1.33]	1.11 [0.80–1.55]	1.10 [0.87–1.38]	0.56	0.94
<i>DRB1*07:01</i>	13.3	1.04 [0.89–1.23]	1.12 [0.84–1.49]	1.01 [0.83–1.23]	0.73	0.58
<i>DRB1*15:01</i>	12.5	0.88 [0.74–1.04]	0.88 [0.65–1.20]	0.88 [0.72–1.07]	0.35	0.98
<i>DQA1*01:01</i>	15.1	0.91 [0.78–1.06]	0.97 [0.72–1.31]	0.88 [0.73–1.06]	0.44	0.59
<i>DQA1*01:02</i>	18.4	0.91 [0.79–1.05]	0.95 [0.73–1.23]	0.90 [0.76–1.06]	0.45	0.73
<i>DQA1*01:03</i>	6.8	0.81 [0.65–1.01]	0.75 [0.49–1.16]	0.83 [0.64–1.07]	0.17	0.71
<i>DQA1*02:01</i>	13.4	1.04 [0.88–1.22]	1.11 [0.83–1.49]	1.01 [0.83–1.23]	0.74	0.57
<i>DQA1*03:01</i>	18.6	1.09 [0.95–1.26]	1.06 [0.82–1.36]	1.11 [0.93–1.32]	0.41	0.78
<i>DQA1*05:01</i>	25.1	1.12 [0.99–1.27]	1.04 [0.83–1.30]	1.16 (1.00–1.34)	0.12	0.42
<i>DQB1*02:01</i>	17.8	1.06 [0.92–1.22]	1.09 [0.85–1.41]	1.05 [0.89–1.24]	0.63	0.78
<i>DQB1*03:01</i>	19.1	1.12 [0.98–1.28]	1.07 [0.84–1.38]	1.14 [0.97–1.33]	0.23	0.72
<i>DQB1*03:02</i>	10.6	1.08 [0.91–1.29]	1.18 [0.86–1.61]	1.04 [0.85–1.29]	0.53	0.54
<i>DQB1*05:01</i>	12.3	0.89 [0.75–1.06]	0.98 [0.71–1.35]	0.86 [0.70–1.06]	0.38	0.51
<i>DQB1*06:02</i>	12.0	0.90 [0.76–1.06]	0.92 [0.67–1.25]	0.89 [0.73–1.09]	0.48	0.87
<i>DQB1*06:03</i>	6.3	0.80 [0.64–1.01]	0.79 [0.51–1.23]	0.81 [0.62–1.06]	0.20	0.93
<i>DPA1*01:03</i>	18.8	0.94 [0.80–1.10]	0.92 [0.70–1.21]	0.95 [0.78–1.15]	0.75	0.86
<i>DPA1*02:01</i>	14.9	0.96 [0.81–1.14]	1.06 [0.79–1.43]	0.92 [0.75–1.12]	0.66	0.42
<i>DPB1*02:01</i>	13.4	1.19 (1.02–1.39)	1.02 [0.76–1.38]	1.25 (1.05–1.50)	0.04	0.25
<i>DPB1*03:01</i>	10.3	0.80 (0.65–0.97)	1.08 [0.77–1.49]	0.67 (0.53–0.86)	0.007	0.03
<i>DPB1*04:01</i>	42.4	1.03 [0.92–1.15]	1.02 [0.83–1.25]	1.03 [0.90–1.18]	0.87	0.94
<i>DPB1*04:02</i>	11.1	1.01 [0.85–1.21]	0.94 [0.68–1.29]	1.05 [0.84–1.30]	0.84	0.58
rs9273349 T	40.8	0.82 (0.72–0.93)	0.81 [0.64–1.03]	0.82 (0.71–0.95)	0.009	0.95

Data are presented as OR (95% CI) unless otherwise stated. AF: allele frequency. [#]: weighted average in ESE and B58C; [†]: n=5079; ⁺: n=1683; [§]: n=3396. Bold face indicates p<0.05.

Association between rs9273349 and adult-onset asthma was similar in exposed and unexposed individuals (test for gene–environment interaction p=0.95). For *DPB1*03:01*, the joint test was statistically significant (p=0.007, interaction p=0.03). In unexposed individuals, *DPB1*03:01* was associated with a decreased risk of adult-onset asthma (OR 0.67, p=0.002), but not in exposed subjects (OR 1.08, p=0.64). Study-specific estimates showed that the interaction was driven by the ESE cohorts (OR for exposed 1.34, p=0.20; OR for unexposed 0.62, p=0.0009). In ESE subjects carrying at least one *DPB1*03:01* allele, the proportion of subjects with adult-onset asthma was higher in those exposed to occupational HMW allergens (35.6%) and lower in the unexposed (15.0%), compared with the same groups in ESE noncarriers (28.4% and 22.2% in exposed and unexposed, respectively; fig. E1). Thus, in these cohorts, a stronger association between adult-onset asthma and occupational HMW allergens was detected in carriers of at least one *DPB1*03:01* allele (OR 3.13, 95% CI 1.87–5.23) than in noncarriers (OR 1.39, 95% CI 1.08–1.81). In B58C, *DPB1*03:01* was not associated with asthma, whichever the exposure status (OR exposed 0.84, p=0.48; OR unexposed 0.89, p=0.62). Associations of *DPB1*03:01* and rs9273349 with adult-onset asthma were independent: the effect of each genetic polymorphism did not change when both were jointly included in a multiple regression model.

In the HMW allergen exposed industrial cohorts (meta-analysis of AGRI and BAKERY), *DPB1*03:01* was not associated with asthma (table 3). However, the test for gene–environment interaction between *DPB1*03:01* and HMW exposure was more significant after including the industrial cohorts together with the population-based cohorts in the analysis (p=0.009). The association of allele *DQB1*06:02* with asthma (p=0.007) was restricted to the industrial cohorts, with a similar effect in agricultural workers (OR 0.72, p=0.05) and bakery workers (OR 0.65, p=0.07).

As the majority of exposed subjects from the general population cohorts were exposed to latex, an additional sensitivity analysis was performed in ESE and B58C, by testing for gene–environment interaction in two mutually exclusive exposed groups: “ever being exposed to latex” and “ever being exposed to HMW allergens, but never to latex”, with unexposed as a reference group (tables E4 and E5). The gene–environment interaction between *DPB1*03:01* and overall HMW exposure was only observed in latex

TABLE 3 Association of imputed classical human leukocyte antigen (HLA) class II alleles, rs9273349, and adult-onset asthma in the industrial cohorts of agricultural workers and bakery workers, all subjects ever exposed to occupational high molecular weight (HMW) allergens (industrial cohorts, ESE and B58C) and unexposed subjects

HLA-II allele	AF [#]	Industrial HMW allergen exposed only [†]	All HMW allergen exposed [†]	HMW allergen unexposed [‡]	p-value	
					Joint test	Interaction
<i>DRB1*01:01</i>	9.4	0.96 (0.70–1.31)	0.90 (0.71–1.15)	0.83 (0.66–1.04)	0.21	0.62
<i>DRB1*03:01</i>	13.0	1.30 (1.01–1.68)	1.18 (0.97–1.43)	1.11 (0.91–1.36)	0.12	0.68
<i>DRB1*04:01</i>	10.0	0.93 (0.68–1.27)	1.01 (0.80–1.27)	1.10 (0.87–1.38)	0.70	0.61
<i>DRB1*07:01</i>	12.8	0.96 (0.70–1.32)	1.04 (0.84–1.29)	1.01 (0.83–1.23)	0.90	0.84
<i>DRB1*15:01</i>	13.1	0.73 (0.57–0.94)	0.78 (0.65–0.95)	0.88 (0.72–1.07)	0.03	0.43
<i>DQA1*01:01</i>	14.9	1.08 (0.83–1.42)	1.03 (0.84–1.26)	0.88 (0.73–1.06)	0.42	0.27
<i>DQA1*01:02</i>	19.1	0.77 (0.62–0.95)	0.83 (0.71–0.99)	0.90 (0.76–1.06)	0.06	0.53
<i>DQA1*01:03</i>	6.9	1.20 (0.84–1.73)	0.99 (0.75–1.31)	0.83 (0.64–1.07)	0.38	0.35
<i>DQA1*02:01</i>	12.9	0.98 (0.71–1.34)	1.05 (0.85–1.30)	1.01 (0.83–1.23)	0.89	0.79
<i>DQA1*03:01</i>	18.4	0.87 (0.68–1.12)	0.96 (0.80–1.15)	1.11 (0.93–1.32)	0.44	0.26
<i>DQA1*05:01</i>	25.1	1.31 (1.06–1.62)	1.17 (1.01–1.37)	1.16 (1.00–1.34)	0.01	0.91
<i>DQB1*02:01</i>	18.3	1.14 (0.91–1.43)	1.12 (0.95–1.32)	1.05 (0.89–1.24)	0.33	0.58
<i>DQB1*03:01</i>	18.4	1.06 (0.82–1.36)	1.06 (0.89–1.27)	1.14 (0.97–1.33)	0.21	0.59
<i>DQB1*03:02</i>	10.7	0.86 (0.63–1.17)	1.00 (0.80–1.25)	1.04 (0.85–1.29)	0.90	0.79
<i>DQB1*05:01</i>	12.1	1.03 (0.76–1.39)	1.00 (0.80–1.25)	0.86 (0.70–1.06)	0.38	0.32
<i>DQB1*06:02</i>	12.6	0.70 (0.54–0.91)	0.78 (0.64–0.95)	0.89 (0.73–1.09)	0.03	0.37
<i>DQB1*06:03</i>	6.4	1.19 (0.82–1.73)	1.00 (0.75–1.33)	0.81 (0.62–1.06)	0.33	0.29
<i>DPA1*01:03</i>	18.7	1.08 (0.84–1.38)	1.00 (0.83–1.20)	0.95 (0.78–1.15)	0.88	0.69
<i>DPA1*02:01</i>	14.8	1.04 (0.79–1.37)	1.05 (0.86–1.28)	0.92 (0.75–1.12)	0.64	0.35
<i>DPB1*02:01</i>	13.3	1.11 (0.84–1.46)	1.07 (0.87–1.30)	1.25 (1.05–1.50)	0.03	0.24
<i>DPB1*03:01</i>	10.4	1.02 (0.76–1.38)	1.04 (0.84–1.30)	0.67 (0.53–0.86)	0.007	0.009
<i>DPB1*04:01</i>	42.4	0.84 (0.69–1.01)	0.91 (0.79–1.05)	1.03 (0.90–1.18)	0.42	0.22
<i>DPB1*04:02</i>	11.2	1.37 (1.02–1.83)	1.15 (0.93–1.42)	1.05 (0.84–1.30)	0.38	0.55
rs9273349 T	41.4	0.89 (0.74–1.07)	0.86 (0.74–0.99)	0.82 (0.71–0.95)	0.005	0.69

Data are presented as OR (95% CI) unless otherwise stated. AF: allele frequency. [#]: weighted average in industrial cohorts, ESE and B58C; [†]: n=946; [‡]: n=2629; [§]: n=3396. Bold face indicates p<0.05.

exposed *versus* unexposed subjects (interaction p=0.01). As a result, in ESE subjects carrying at least one *DPB1*03:01* allele, a strong association between latex exposure and adult-onset asthma was observed (OR 3.12, 95% CI 1.73–5.65).

Association between the 18 tag SNPs in the HLA-II region and adult-onset asthma was similar in occupationally exposed and unexposed individuals (interaction p>0.05; table E6).

Discussion

In a meta-analysis of European cohorts, we did not find evidence to suggest that common HLA-II alleles are associated with the risk of adult-onset asthma. However, when analyses were stratified by occupational HMW allergen exposure, the *DPB1*03:01* allele appeared to be negatively associated with adult-onset asthma in unexposed subjects, and an interaction was found between *DPB1*03:01* and exposure to latex. *DQB1*06:02* showed a consistent inverse association with asthma in cohorts of bakery workers and agricultural workers, but not in the HMW allergen exposed subjects from the general population studies.

We showed that rs9273349, the SNP in the *HLA-DQ* region that emerged from the GABRIEL GWAS [4], influences adult-onset asthma to an equal extent in both HMW allergen exposed and occupationally unexposed individuals. Thus, no evidence was found to suggest that occupational allergen exposures might be involved in the association of rs9273349, or any of the other 18 selected tag SNPs in the HLA-II region, and adult-onset asthma. rs9273349 was significantly associated with adult-onset asthma in combined ESE and B58C studies (OR 0.82, p=0.002). We also computed this association in the other GABRIEL adult cohorts that were not included in the present study because detailed occupational history information was not available, showing a similar result (OR 0.80, p=0.001). We have shown that rs9273349 is in a region of extended linkage disequilibrium (as indicated by D') that includes *DRB1*, *DQA1* and *DQB1*, but not *DPA1* or *DPB1*. However, none of the classical HLA-II alleles were strongly correlated with rs9273349 (r²=0.00–0.32; tables E2 and E3), and the rs9273349 association signal was not explained by the classical HLA-II alleles in

multiple regression models. Despite the well described role of HLA-II molecules in allergen recognition, it is as yet unclear what the association of rs9273349 with adult-onset asthma might mean. It could be speculated that this SNP might influence the level of expression of HLA molecules. Alternatively, non-HLA genes could play a role. The MHC is one of the most complicated regions of the human genome, with extensive linkage disequilibrium that extends across hundreds of HLA and non-HLA genes. Linkage and association between HLA variants and asthma may in part be due to association between nearby non-HLA genes (in linkage disequilibrium with HLA) and asthma, e.g. *TNFA* [33]. The latter explanation would imply a role for different mechanisms, which may also explain why we did not find an interaction between rs9273349 and occupational allergen exposure.

Our study population was large enough to reveal moderate associations between classical HLA-II alleles and adult-onset asthma, and the complete absence of marginal associations is probably not due to a lack of statistical power. When taking occupational exposure into account, carrying *DPB1*03:01* appeared to protect against adult-onset asthma among unexposed individuals. However, in ESE subjects carrying the *DPB1*03:01* allele, a strong positive association between exposure to latex and adult-onset asthma was observed (OR >3). This finding suggests that *DPB1*03:01* may confer an increased risk of developing asthma in adulthood, but only for those with specific workplace exposures. We can only speculate on the mechanism behind an opposite effect among the unexposed. A better protection against respiratory infections or the development of allergy to other antigens may be involved. A German case-control study investigated the role of *HLA-DQ* and *HLA-DR* (but not *HLA-DP*) among 269 health care workers with latex allergy and 90 nonatopic control subjects [34]. *DRB1*04/DQB1*03:02* haplotype was associated with an increased risk of IgE to a major latex allergen (Hev b 6.02) [34]. We also found positive, but not significant, associations of *DRB1*04:01* and *DQB1*03:02* with adult-onset asthma among our 1123 latex-exposed workers. Only a few other studies investigated HLA alleles in relation to asthma or allergic outcomes among workers exposed to HMW allergens, namely laboratory animal allergens [35], cow dander [36] and fish food [37]. *DQB1*06:02*, which was here associated with asthma both in bakery workers and agricultural workers, did not emerge from these other studies.

Studying gene-environment interaction in large-scale studies has some inherent limitations. In populations sampled from the general population (or asthma patients and their families as in EGEA), there is a large heterogeneity in occupations and related exposures. The majority of workers who were classified as “exposed to HMW agents” are latex-exposed healthcare professionals who are typically exposed to other agents, such as cleaning products, that may cause asthma through a non-IgE mediated mechanism [38]. Despite the heterogeneity in exposures, associations between occupational exposure to HMW allergens and adult-onset asthma were comparable across cohorts and showed a significantly increased risk, which was similar in magnitude to earlier published associations [15, 16, 19]. In the present sample of B58C, the association of HMW exposure and asthma appeared to be weaker than the earlier published association in a larger sample of B58C [16]. This difference appeared to be due to chance variation, as the odds ratios did not differ significantly between the B58C samples with and without genotyping available.

The proportion of males among exposed subjects was lower in SAPALDIA (12%) and ECRHS (14%) than in EGEA (29%) and B58C (33%). In the ESE exposed group, association between *DPB1*03:01* and adult-onset asthma was more pronounced in males (OR 2.71, $p=0.055$) than in females (OR 1.18, $p=0.52$), but interaction was not significant ($p=0.18$) due to small sample size. This association did not differ between unexposed males and females in ESE. The workforce-based studies also differed from the population-based cohorts in terms of exposures, phenotype and proportion of male subjects. Therefore, we performed separate analyses in population-based cohorts only and in all exposed subjects. Also within the workforce-based cohorts, heterogeneity in source, nature and levels of allergen exposures exists. Bakery workers can be exposed to allergenic enzymes and flour allergens, but also to cleaning products, whereas agricultural workers (e.g. grain farmers and dairy farmers) may be exposed to allergens from animal danders, storage mites, grain and flour. Various other exposures in the agricultural environment, such as endotoxins and disinfectants, are also associated with (nonallergic) asthma. The association of *DQB1*06:02* with asthma among bakers and farmers may provide further explanations why some of these workers develop respiratory health problems, while others with similar exposure levels remain healthy [39].

In this large-scale meta-analysis, we were not able to study specific IgE to occupational allergens (e.g. latex, wheat and α -amylase) or clinically assessed occupational asthma as a phenotype. Instead, we used adult-onset asthma, which was consistently associated with HMW allergen exposure (fig. 1) and with rs9273349 in GWAS. We aimed to further investigate this HLA signal in GWAS and, therefore, we decided not to include other asthma phenotypes or atopic sensitisation in the present study. Although a substantial part of adult-onset asthma is attributable to occupational exposures, other risk factors, triggers and comorbid conditions such as smoking, female sex hormones, respiratory infections and obesity are also associated

with the development of asthma in adulthood [40]. The relevance of HLA-II to most of these determinants is, however, less obvious.

In conclusion, imputed common HLA-II alleles were not associated *per se* with adult-onset asthma in a meta-analysis of European cohorts. However, when taking occupational allergen exposures into account, results showed a negative association between *DPB1*03:01* and adult-asthma in the unexposed, and suggested a gene–environment interaction between the *DPB1*03:01* allele and exposure to latex. Furthermore, *DQB1*06:02* was associated with asthma among bakery workers and agricultural workers, suggesting a role for specific occupational allergens. Occupational HMW allergen exposures did not modify the association between rs9273349, the SNP in the *HLA-DQ* region that emerged from the GABRIEL GWAS, and adult-onset asthma.

Acknowledgements

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We are grateful to all study members and staff involved in data collections in each cohort. For the 1958 Birth Cohort, the asthma-specific job–exposure matrix was applied with an expert re-evaluation step by UK experts in occupational lung disease (Paul Cullinan, David Fishwick (Centre for Workplace Health, Sheffield, UK), Jennifer Hoyle (North Manchester General Hospital, Manchester, UK) and Chris Warburton (North Manchester General Hospital, Manchester, UK)). Further acknowledgements are given in the online supplementary material.

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ONLINE SUPPLEMENT

HLA-II variants and adult-onset asthma: does occupational allergen exposure play a role?

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SUPPLEMENTARY METHODS

Cohort descriptions

B58C

The British 1958 birth cohort is an ongoing follow-up of all persons born in England, Scotland and Wales during one week in 1958. Lifetime occupational histories for cohort members were derived from interviews of cohort members at ages 33 and 42 years, as described in detail elsewhere [E1,E2]. Jobs were coded to the International Standard Classification of Occupations 1988 from job titles, a short free text job description and an Asthma Specific Job Exposure Matrix (ASJEM) with an expert re-evaluation step by UK experts in occupational lung disease (Paul Cullinan, David Fishwick, Jennifer Hoyle, Chris Warburton) [E1].

For the purposes of this gene-environment interaction analysis, the reference (non-exposed) group was defined as cohort members who, by age 42 had only ever worked in an occupation that was considered by the ASJEM to be “non-exposed”. Two mutually exclusive exposed groups were defined: “HMW exposed” comprised participants who had ever worked in a job associated with high molecular weight allergen exposures, with or without other occupational exposures associated with asthma risk. “Other exposed” comprised cohort members who had never worked in occupations associated with HMW allergen exposure, but had worked at some time in jobs identified by ASJEM as “exposed”. These “other exposed” individuals were excluded from the gene-environment interaction analysis within the HLA region.

Asthma and wheezing were defined prospectively from parental interviews when the cohort members were aged 7, 11 and 16 years of age, and from interviews with participants at 23, 33 and 42 years of age, as detailed elsewhere [E3]. For the purposes of this gene-environment interaction analysis, adult-onset asthma was defined as a lifetime history of asthma by age 42, in the absence of parental reports of asthma or wheezy bronchitis at age 7 or 11 or 16.

B58C Genotyping

At the age of 44-45 years, the cohort were followed up with a biomedical examination and blood sampling [E4], from which a DNA collection was established as a nationally representative reference panel. Non-overlapping subsets of the DNA collection were genotyped by the Wellcome Trust Case-Control Consortium (WTCCC) [E5], the Type 1 Diabetes Genetics Consortium (T1DGC) [E6] and the GABRIEL consortium [E7]. Genotyping by the WTCCC used both the Affymetrix 500K array and the Illumina 550K (version 1) array. Since the T1DGC used the Illumina 550K (version 3) array and GABRIEL used the Illumina 610K array, a combined dataset was created of SNPs in common across these three panels. SNPs were excluded from subsequent imputation if they had $MAF < 1\%$, $call-rate < 95\%$, $HWE\ p-value < 0.0001$, or differences in allele frequency across the three deposits ($p < 0.0001$ on pairwise comparisons). Imputation of markers in the HLA region which were genotyped on the 610K array but not present on the 550K array was performed using MACH [E8]. More extensive imputations across the MHC region and derivation of HLA variants were performed as described for the other cohorts.

Within-cohort associations of genotyped data and imputed allele dosages with asthma outcomes among exposed and non-exposed groups were analysed using ProbABEL [E9] and Stata version 10.

ECRHS

The methodology of ECRHS has been described elsewhere [E10,E11]. Briefly, the ECRHS is a random population-based multicentre cohort from sixteen centers (eight countries with DNA samples) of subjects aged 20-44 at time of recruitment (1990, ECRHS-I) and then followed-up approximately 10 years later (ECRHS-II). In each centre, a representative community-based sample of at least 3000 adults aged 20-44 years were invited to complete a

brief postal questionnaire asking about respiratory symptoms (ECRHS I - Stage 1) between 1991-1993. A random sample of these (600 per centre) underwent intensive further investigation (ECRHS I - Stage 2 – random sample). Participants who had symptoms highly suggestive of asthma but who had not been selected at random to take part in Stage 2 were also invited to undergo intensive investigations (ECRHS I - Stage 2- enriched sample). About ten years later all adults who had taken part in Stage 2 were re-contacted (ECRHS II) and again asked about respiratory symptoms. Samples suitable for DNA extraction were collected. All cases of asthma were genotyped (participants from the random or enriched sample who said yes to the question ‘Have you ever had asthma?’ at either ECRHS I Stage 2 or at ECRHS II). Controls were a random sample (of the random sample) who answered ‘no’ to the same question in both surveys. Subjects reported their complete job history. For the purposes of this gene-environment interaction analysis, the job history between ECRHS I and ECRHS II was considered. Jobs were coded to the ISCO88 from job titles, a short free text job description, and the ASJEM with an expert re-evaluation step (by trained national experts) was applied [E12].

EGEA

EGEA is a 12-year longitudinal survey which combines a case-control study and a family study. The first survey (EGEA1) took place between 1992 and 1995 [E13,E14]. The study population included 388 asthmatics recruited in chest clinics and their 1,244 family members plus 415 population-based controls (total of 2,047 subjects). The probands (asthmatics and controls) were between 7 and 70 years old at the time of the study. All probands and their two parents were of European ancestry and were born in France. The second survey (EGEA2) was conducted between 2003 and 2007 and included follow-up data in 1,543 subjects from the initial cohort and 73 new family members. Data was collected through face-to-face

interviews and clinical examination. For the purposes of this gene-environment interaction analysis, ever asthma was defined as “Have you ever had attacks of breathlessness at rest with wheezing?” and/or “Have you ever had asthma attacks?” at EGEA1 or EGEA2 and/or being recruited as an asthma cases in a chest clinic. Lifetime occupational history was available at EGEA2 for most participants, the last two jobs at EGEA1 or EGEA2 for others. All jobs and training periods were coded to the ISCO88 from job titles, a detailed task job description, and the ASJEM with an expert re-evaluation step (Dick Heederik, Michel Héry, Nicole Le Moual) was applied [E15].

SAPALDIA

SAPALDIA is a population-based cohort that originally recruited subjects aged 18 to 60 from population registries in eight Swiss communities representing the three largest language groups (German, French, Italian) as well as different levels of air pollution, altitude and degrees of urbanization [E16,E17]. All self-reported asthma cases as well as a random sample of controls were genotyped. These subjects were obtained from among 6,055 SAPALDIA cohort subjects that participated in both the baseline (1991) and follow-up (2002) examinations and agreed to providing blood for genetic analysis. At both baseline and follow-up examination subjects underwent spirometry as well as a detailed interview on respiratory health, smoking history, lifestyle factors and anthropometry. At follow-up, 8,047 of 9,651 baseline subjects re-participated in at least one part of the study and a formal biobank was established. Asthma status was defined by an affirmative answer to the question “Have you ever had asthma” at baseline and/or follow-up interview. For the purposes of this gene-environment interaction analysis, job history during the period between SAPALDIA-1 and SAPALDIA-2, and/or reported at the time of SAPALDIA-1 was considered. All jobs were

coded to the ISCO88 from job titles (Dirk Keidel, Amar Mehta, Nicole Le Moual) and the ASJEM with an expert re-evaluation step (David Miedinger, David Vernez) was applied.

Industrial Cohorts

The industrial cohorts comprise several pooled surveys on occupational asthma from Denmark and The Netherlands. Cohorts of agricultural workers ('AGRI') included the Danish SUS study, the Dutch Omega study, and the Dutch Veterinarian's Health Study [E18]. Bakery workers ('BAKERY') were sampled from the Dutch Bakers health surveillance project [E19]. All subjects were classified as exposed to high molecular weight sensitizers (flour, enzymes, animal proteins, mites or plant antigens). In the industrial cohorts, asthma was defined as one or more current asthma symptoms. Current asthma cases and controls (matched for study, industry, gender, and age) were genotyped. Asthma symptoms were associated with workplace exposures [E18, E19], and were therefore included in the analysis on adult-onset asthma. Current asthma was defined as a positive question to at least one of the following questions: 1) "Have you had wheezing or whistling in your chest at any time in the last 12 months?", 2) "Have you had an attack of asthma in the last 12 months?", 3) "Have you been woken by an attack of shortness of breath at any time in the last 12 months?", or 4) "Are you currently taking any medicine for asthma?".

Selection of tag SNPs

We investigated whether the association of the GABRIEL main signal (rs9273349) with adult-onset asthma was modified by occupational exposure. For completeness, we further selected 37 SNPs belonging to HLA-II region (from 32,515,515 bp to 33,204,860 bp +/-50 kb (build 36.3)) that were associated with asthma and/or adult-onset asthma and/or industrial asthma at p-value $\leq 10^{-4}$ in GABRIEL asthma GWAS. Among those SNPs, 35 fulfilled the

quality control criteria in our populations under study (i.e., Hardy-Weinberg p -value $\geq 10^{-4}$ among controls, call rate $\geq 97\%$, and minor allele frequency $\geq 5\%$). We selected a subset of 18 tag SNPs using Haploview [E20], and tested for gene-environment interaction with occupational exposure to HMW allergens.

Occupational exposure to HMW allergens

Participants of ESE and B58C cohorts reported at least their two most recent jobs, which were coded to the international standard classification of occupations (ISCO88). In all cohorts, occupations were linked to an asthma-specific job-exposure matrix (JEM) that classifies occupational exposure to 18 high-risk substances [E21]. In the present study, we only considered seven HMW high-risk substances (namely animal proteins, fish or shellfish, mites, latex, flour, other plant antigens, and enzymes) that may cause asthma through a similar, predominantly IgE-associated mechanism; we did not consider other high-risk substances (i.e. low molecular-weight (LMW) substances, bioaerosols, pharmaceutical drugs, mixed environments, and irritant peaks), because these have not, or not consistently, been associated with IgE-associated allergy. All other jobs were classified as ‘low risk’ or ‘non-exposed’. This JEM favors specificity over sensitivity: occupations were classified as exposed only if the probability of exposure was expected to be high. The validity of the exposure assignment was enhanced by local expert re-evaluation of exposure estimates (‘expert judgement step’) [E21]. Subjects who were ever exposed to any of the seven HMW allergens were classified as ‘exposed’. The ‘non-exposed’ reference group consisted of subjects who were never exposed to any of the high-risk or low-risk jobs. Subjects who were never exposed to any of the seven HMW allergens, but ever exposed to the other high-risk substances or low-risk jobs, were excluded from data analysis. Subjects who were non-exposed according to the JEM, but who completed a job-specific questionnaire for healthcare

workers or cleaners (ESE cohorts only) were excluded from data analysis because of potential exposure to LMW substances. The agricultural workers and bakery workers (industrial cohorts) were all classified as exposed to HMW allergens.

Statistical analysis

First, we aimed to confirm the earlier published association of exposure to HMW allergens with adult-onset asthma in the samples from EGEA, SAPALDIA, ECRHS, and B58C that were used in the present study. The association was assessed by means of logistic regression analysis, adjusted for sex, age, and smoking status. In ECRHS, the association was also adjusted for study center as random intercept. To account for familial dependence in EGEA, we estimated ORs and 95% confidence intervals (CIs) by generalized estimating equations using family as a cluster. We assumed an exchangeable correlation structure in which the observations within a family are assumed to be equally correlated. Study-specific estimates were combined through fixed effects meta-analysis.

In subsequent genetic analyses, the ESE cohorts were pooled to increase statistical power and to avoid convergence problems, as was done before with EGEA and ECRHS data [E22]. Raw data from B58C were not available for pooled analysis. We tested association of 23 common classical HLA-II alleles with allele frequency >5% in each population with adult-onset asthma, assuming an additive genetic model. Population admixture was taken into account by including principal components for within-Europe diversity in ESE cohorts, as covariate in the association analysis. The PCs were computed on pooled ESE samples using genome-wide genotyping data and EIGENSTRAT2.0 software [E23]. Genetic effects were estimated in each cohort separately, stratified by exposure status (HMW-exposed: ESE, B58C, BAKERY, AGRI; Non-exposed: ESE, B58C). The overall (exposure-adjusted) gene effect was estimated by standard fixed-effects meta-analysis of ESE-exposed, ESE-non-exposed, B58C-exposed,

and B58C-non-exposed. Consistency of results was verified by assessing heterogeneity of genetic effects across the different cohorts. To evaluate gene-environment interaction, results in exposed subjects and non-exposed subjects from ESE and B58C were combined through fixed-effects meta-analysis to obtain OR in exposed and non-exposed groups. Both the standard one-degree-of-freedom test of gene-environment interaction and the two-degree-of-freedom joint test of marginal genetic effects and gene-environment interaction were performed, the latter being generally considered to have greater power than the marginal test when the genetic effect is only expressed in exposed individuals [E24, E25].

Additional analyses were done by including the industrial cohorts in the meta-analysis of HMW-exposed workers, and performing the joint test and test for interaction. The industrial cohorts were not included to evaluate the marginal genetic effect because they are exposed-only, so the interaction effect cannot be assumed to be independent of the exposed-only OR.

Furthermore, gene-environment interaction between rs9273349 and the 18 tag SNPs in the HLA-II region and HMW allergen exposure was tested.

We did not adjust for multiple testing because of the prior evidence of HLA-II involvement in several GWAS. Instead, we used a p-value threshold of $p < 0.01$ for the test of marginal association, joint test and test for interaction.

Funding

B58C

We acknowledge the original creators, sponsors, depositors and distributors of the British 1958 birth cohort data. Asthma UK provided funding for the ASJEM coding of occupational exposures and investigation of their associations with asthma and the COLT Foundation provided funding for extension of this work to look at other allergic outcomes and completion of a PhD by RE Ghosh. We acknowledge use of phenotype and genotype data from the British 1958 Birth Cohort DNA collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02. (<http://www.b58cgene.sgul.ac.uk/>). Genotyping for the B58C-WTCCC subset was funded by the Wellcome Trust grant 076113/B/04/Z. The B58C-T1DGC genotyping utilized resources provided by the Type 1 Diabetes Genetics Consortium, a collaborative clinical study sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), National Human Genome Research Institute (NHGRI), National Institute of Child Health and Human Development (NICHD), and Juvenile Diabetes Research Foundation International (JDRF) and supported by U01 DK062418. B58C-T1DGC GWAS data were deposited by the Diabetes and Inflammation Laboratory, Cambridge Institute for Medical Research (CIMR), University of Cambridge, which is funded by 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 B58C-GABRIEL genotyping was supported by a contract from the European Commission Framework Programme 6 (018996) and grants from the French Ministry of Research.

ECRHS was supported by the European Commission, as part of their Quality of Life program. The coordination of ECRHS II was supported by the European Commission, as part of their Quality of Life program. The following bodies funded the local studies in ECRHS II in this article: **Albacete**-Fondo de Investigaciones Santarias (grant code: 97/0035-01, 99/0034-01, and 99/0034-02), Hospital Universitario de Albacete, Consejería de Sanidad. **Antwerp**-FWO (Fund for Scientific Research)-Flanders Belgium (grant code: G.0402.00), University of Antwerp, Flemish Health Ministry. **Barcelona**-Fondo de Investigaciones Sanitarias (grant code: 99/0034-01, and 99/0034-02), Red Respira (RTIC 03/11 ISC IIF). Ciber of Epidemiology and Public Health has been established and founded by Instituto de Salud Carlos III. **Erfurt**-GSF–National Research Centre for Environment & Health, Deutsche Forschungsgemeinschaft (DFG) (grant code FR 1526/1-1). **Galdakao**-Basque Health Department. **Grenoble**-Programme Hospitalier de Recherche Clinique-DRC de Grenoble 2000 no.2610, Ministry of Health, Direction de la Recherche Clinique, Ministère de l'Emploi et de la Solidarité, Direction Générale de la Santé, CHU de Grenoble, Comité des Maladies Respiratoires de l'Isère. **Ipswich and Norwich**-National Asthma Campaign (UK). **Huelva**-Fondo de Investigaciones Sanitarias (FIS) (grant code: 97/0035-01, 99/0034-01, and 99/0034-02). **Oviedo**-Fondo de Investigaciones Santarias (FIS) (grant code: 97/0035-01, 99/0034-01, and 99/0034-02). **Paris**-Ministère de l'Emploi et de la Solidarité, Direction Générale de la Santé, UCBPharma (France), Aventis (France), Glaxo France, Programme Hospitalier de Recherche Clinique-DRC de Grenoble 2000 no. 2610, Ministry of Health, Direction de la Recherche Clinique, CHU de Grenoble. **Pavia**-Glaxo, Smith & Kline Italy, Italian Ministry of University and Scientific and Technological Research (MURST), Local University Funding for Research 1998 & 1999 (Pavia, Italy). **Turin**-ASL 4 Regione Piemonte (Italy), AO CTO/ICORMA Regione Piemonte (Italy), Ministero dell'Università e della Ricerca Scientifica (Italy), Glaxo Wellcome spa (Verona, Italy). **Umeå**-Swedish Heart

Lung Foundation, Swedish Foundation for Health Care Sciences & Allergy Research, Swedish Asthma & Allergy Foundation, Swedish Cancer & Allergy Foundation. **Verona-**University of Verona; Italian Ministry of University and Scientific and Technological Research (MURST); Glaxo, Smith & Kline Italy.

EGEA is funded in part by PHRC-Paris, PHRC-Grenoble, ANR 05-SEST-020-02/05-9-97, ANR-06-CEBS, ANR-CES-2009, Région Nord Pas-de-Calais, Merck Sharp & Dohme (MSD)

SAPALDIA received funds from the Swiss National Science Foundation (grants no 33CSCO-108796, 3247BO-104283, 3247BO-104288, 3247BO-104284, 3247-065896, 3100-059302, 3200-052720, 3200-042532, 4026-028099), the Federal Office for Forest, Environment and Landscape, the Federal Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais, and Zürich, the Swiss Lung League, the canton's Lung League of Basel Stadt/ Basel Landschaft, Geneva, Ticino, Valais and Zurich, SUVA, Freiwillige Akademische Gesellschaft, UBS Wealth Foundation, Talecris Biotherapeutics GmbH, Abbott Diagnostics, European Commission 018996 (GABRIEL), Wellcome Trust WT 084703MA. None of the funders had any role in study design, data collection and analysis, or preparation of the manuscript.

Industrial cohorts received funds from The Lung Foundation Netherlands; Ceffic, Longrange research initiative, Belgium; the Dutch Ministry of Social Affairs and Employment; The Dutch Product Board for Grains, Seeds and Pulses; TNO Quality of Life; The Danish Agency for Science Technology and Innovation, The Danish Medical Research

Council, The Danish Agricultural Research Council, Helsefonden, The P.C. Petersen Foundation, The Danish Working Environment Research Fund, The Danish Research Council Aarhus University and The Danish Lung Association. The Danish Work Environment Foundation; Viborg County; The Danish Medical Research Council; The Health Insurance Fund; The Danish Lung Association.

Acknowledgements

We also thank all study members and staff involved in data collections in each cohort:

ECRHS

The ECRHS data incorporated in this analysis would not have been available without the collaboration of the following individuals and their research teams.

ECRHS Co-ordinating centre: P Burney, D Jarvis, S Chinn, J Knox (ECRHS II), C Luczynska+, J Potts.

Steering Committee for ECRHS II: P Burney, D Jarvis, S Chinn, JM Anto, I Cerveri, R de Marco, T Gislason, J Heinrich, C Janson, N Kunzli, B Leynaert, F Neukirch, T Rochat, J Schouten, J Sunyer; C Svanes, P Vermeire+, M Wjst.

Principal Investigators and Senior Scientific Teams for ECRHS II: Australia: Melbourne (M Abramson, R Woods, EH Walters, F Thien), Belgium: South Antwerp & Antwerp City (P Vermeire+, J Weyler, M Van Sprundel, V Nelen), Denmark: Aarhus (EJ Jensen), Estonia: Tartu (R Jogi, A Soon), France: Paris (F Neukirch, B Leynaert, R Liard, M Zureik), Grenoble (I Pin, J Ferran-Quentin), Bordeaux (A Taytard, C Raherison), Montpellier (J Bousquet, P Demoly), Germany: Erfurt (J Heinrich, M Wjst, C Frye, I Meyer), Hamburg (K Richter, D Nowak, K Radon), Iceland: Reykjavik (T Gislason, E Bjornsson, D Gislason, T Blondal, A Karlsdottir), Italy: Turin (M Bugiani, P Piccioni, E Caria, A Carosso, E Migliore, G

Castiglioni), Verona (R de Marco, G Verlato, E Zanolin, S Accordini, A Poli, V Lo Cascio, M Ferrari), Pavia (A Marinoni, S Villani, M Ponzio, F Frigerio, M Comelli, M Grassi, I Cerveri, A Corsico), Netherlands: Groningen & Geleen (J Schouten, M Kerkhof), Norway: Bergen (A Gulsvik, E Omenaas, C Svanes, B Laerum), Spain: Barcelona (JM Anto, J Sunyer, M Kogevinas, JP Zock, X Basagana, A Jaen, F Burgos), Huelva (J Maldonado, A Pereira, JL Sanchez), Albacete (J Martinez-Moratalla Rovira, E Almar), Galdakao (N Muniozguren, I Urritia), Oviedo (F Payo), Sweden: Uppsala (C Janson, G Boman, D Norback, M Gunnbjornsdottir), Goteborg (K Toren, L Lillienberg, AC Olin, B Balder, A Pfeifer-Nilsson, R Sundberg), Umea (E Norrman, M Soderberg, K Franklin, B Lundback, B Forsberg, L Nystrom), Switzerland: Basel (N Kunzli, B Dibbert, M Hazenkamp, M Brutsche, U Ackermann-Liebrich), UK: Norwich (D Jarvis, B Harrison), Ipswich (D Jarvis, R Hall, D Seaton), USA: Portland (M Osborne, S Buist, W Vollmer, L Johnson). +Deceased

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Clinical centers: Grenoble: I Pin, C Pison; Lyon: D Ecochard (Egea1), F Gormand, Y Pacheco; Marseille: D Charpin (Egea1), D Vervloet (Egea1-2); Montpellier: J Bousquet; Paris Cochin: A Lockhart (Egea1), R Matran (now in Lille); Paris Necker: E Paty (Egea1-2),

P Scheinmann (Egea1-2); Paris-Trousseau: A Grimfeld (Egea1-2), J Just. **Data and quality management:** Inserm ex-U155 (Egea1): J Hochez; Inserm CESP/U 1018, Villejuif: N Le Moual; Inserm ex-U780: C Ravault (Egea1-2); Inserm ex-U794: N Chateigner (Egea1-2); Grenoble: J Quentin-Ferran (Egea1-2).

The authors thank all those who participated in the setting of the study and on the various aspects of the examinations involved: interviewers, technicians for lung function testing, coders, those involved in quality control and data management, and all those who supervised the study in all centers. The authors are grateful to the 3 CIC-Inserm of Necker, Grenoble and Marseille who supported the study and in which participants were examined. They are indebted to all the participants who participated, without whom that study would not have been possible.

SAPALDIA

Study directorate: T Rochat (p), NM Probst Hensch (e/g), JM Gaspoz (c), N Künzli (e/exp), C Schindler (s).

Scientific team: JC Barthélémy (c), W Berger (g), R Bettschart (p), A Bircher (a), G Bolognini (p), O Brändli (p), C Brombach (n), M Brutsche (p), L Burdet (p), M Frey (p), U Frey (pd), MW Gerbase (p), D Gold (e/c/p), E de Groot (c), W Karrer (p), R Keller (p), B Knöpfli (p), B Martin (pa), D Miedinger (o), U Neu (exp), L Nicod (p), M Pons (p), F Roche (c), T Rothe (p), E Russi (p), P Schmid-Grendelmeyer (a), A Schmidt-Trucksäss (pa), A Turk (p), J Schwartz (e), D. Stolz (p), P Straehl (exp), JM Tschopp (p), A von Eckardstein (cc), E Zemp Stutz (e).

Scientific team at coordinating centers: M Adam (e/g), E Boes (g), PO Bridevaux (p), D Carballo (c), E Corradi (e), I Curjuric (e), J Dratva (e), A Di Pasquale (s), L Grize (s), D Keidel (s), S Kriemler (pa), A Kumar (g), M Imboden (g), N Maire (s), A Mehta (e), F Meier

(e), H Phuleria (exp), E Schaffner (s), GA Thun (g) A Ineichen (exp), M Ragettli (e), M Ritter (exp), T Schikowski (e), G Stern (pd), M Tarantino (s), M Tsai (e), M Wanner (pa).

(a) allergology, (c) cardiology, (cc) clinical chemistry, (e) epidemiology, (exp) exposure, (g) genetic and molecular biology, (m) meteorology, (n) nutrition, (o) occupational health, (p) pneumology, (pa) physical activity, (pd) pediatrics, (s) statistics

The study could not have been done without the help of the study participants, technical and administrative support and the medical teams and field workers at the local study sites.

Local fieldworkers: Aarau: S Brun, G Giger, M Sperisen, M Stahel, Basel: C Bürli, C Dahler, N Oertli, I Harreh, F Karrer, G Novicic, N Wytttenbacher, Davos: A Saner, P Senn, R Winzeler, Geneva: F Bonfils, B Blicharz, C Landolt, J Rochat, Lugano: S Boccia, E Gehrig, MT Mandia, G Solari, B Viscardi, Montana: AP Bieri, C Darioly, M Maire, Payerne: F Ding, P Danieli A Vonnez, Wald: D Bodmer, E Hochstrasser, R Kunz, C Meier, J Rakic, U Schafroth, A Walder.

Administrative staff: C Gabriel, R Gutknecht.

Industrial cohorts

We acknowledge the other team members of the various industrial surveys including: Ø Omland, C Hjort (Danish SUS cohort), E Meijer, E Suarhana (Dutch Bakers Health Surveillance Project), IM Wouters (Dutch VHS and OMEGA study), and G Doekes (Dutch OMEGA study).

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Table E1. Imputation quality of HLA DRB1 and DQB1 alleles in a subset of the B58C study, compared to concordance between imputed data and Dynal genotyping.

HLA-II allele	Imputed allele frequency	Genotype concordance of imputed vs. Dynal (r^2)	Imputation quality (Beagle r^2)
DRB1*01:01	8.2	ND	0.954
DRB1*03:01	14.6	0.919	0.998
DRB1*04:01	11.7	0.815	0.952
DRB1*07:01	13.7	0.919	0.995
DRB1*11:01	3.7	0.372	0.807
DRB1*13:01	4.7	0.536	0.981
DRB1*15:01	13.6	ND	0.998
DQB1*02:01	14.9	ND	0.991
DQB1*03:01	18.4	0.914	0.992
DQB1*03:02	11.2	0.678	0.994
DQB1*05:01	12.2	0.962	0.999
DQB1*06:02	13.3	0.910	0.985
DQB1*06:03	5.0	0.923	0.965

ND: Alleles not determined at 4-digit level by Dynal [E26]. HLA DQA1, DPA1 and DPB1 alleles were also not determined by Dynal. Imputed allele frequencies are based on all B58C subjects included in the present paper. Concordance values are based on 2310 of the B58C subjects included in the present paper, who were previously tested with Dynal and were not part of the reference panel for the Beagle imputations.

Table E2. LD (D') between 19 tag SNPs (selected according to GABRIEL asthma meta-analysis) and 23 HLA class II alleles. LD between HLA-DP alleles and six SNPs could not be computed (NA: not available).

D'	rs3763309	rs9268542	rs2239804	rs13209234	rs34442410	rs28530648	rs9270856	rs9271488	rs3129763	rs35399661	rs9273349	rs9273448	rs9275141	rs5000634	rs1794275	rs3104405	rs9275602	rs241425	rs3132131
DRB1*01:01	0.99 1	0.99 5	0.97 0	0.98 3	0.97 1	0.98 5	0.95 8			0.98 6	0.98 2	0.96 2	0.97 4	0.97 4	0.96 4	0.90 8	0.83 0	0.38 7	0.43 3
DRB1*03:01	0.95 2	0.30 2	0.41 2						0.99 6	0.97 6	0.98 2	0.98 9	0.98 2	0.97 6	0.98 1	0.13 1	0.60 2	0.76 0	0.61 3
DRB1*04:01	0.95 7	0.96 5	0.98 1		0.98 1			0.99 8								0.88 8	0.21 4	0.62 6	0.30 1
DRB1*07:01	0.07 8	0.51 3	0.57 1	0.08 0	0.97 6		0.99 3						0.99 8	0.43 3		0.99 0	0.97 0	0.09 0	0.67 1
DRB1*15:01	0.96 2	0.96 5	0.98 7									0.89 8	0.86 8	0.95 1	0.98 4	0.75 3	0.72 9	0.62 2	0.55 9
DQA1*01:01	0.92 1	0.46 4	0.42 9	0.67 2	0.96 7	0.94 6	0.61 7			0.97 9		0.94 7	0.99 8		0.97 8	0.60 7	0.74 0	0.19 0	0.18 3
DQA1*01:02	0.95 2	0.97 7	0.74 7	0.82 6	0.94 6	0.93 8	0.66 1			0.94 5	0.99 9	0.81 4	0.73 4	0.67 0	0.98 4	0.65 1	0.73 4	0.45 2	0.26 8
DQA1*01:03	0.04 5	0.03 1	0.16 6				0.64 9			0.85 8	0.99 3	0.84 9	0.84 5	0.98 5	0.83 2	0.95 2	0.88 3	0.33 0	0.00 5
DQA1*02:01	0.07 0	0.50 9	0.57 2	0.08 2	0.97 5		0.99 3							0.43 3		0.99 0	0.97 0	0.08 9	0.66 7
DQA1*03:01	0.80 6	0.91 1	0.93 6	0.97 4				0.99 2	0.99 3		0.99 4		0.99 6	0.94 8	0.91 5	0.84 6	0.12 5	0.44 8	0.09 6
DQA1*05:01	0.63 1		0.17 6	0.97 9	0.97 0	0.97 5	0.96 8		0.99 9	0.65 5	0.99 2	0.99 7	0.19 3	0.34 5	0.99 5	0.63 3	0.50 6	0.46 4	0.18 3
DQB1*02:01	0.40 3	0.33 5	0.26 1		0.98 4		0.99 6	0.30 8	0.32 0		0.99 3	0.99 0	0.02 6	0.97 5	0.99 4	0.57 3	0.44 8	0.19 2	0.64 4

DQB1*03:01	0.115	0.766	0.630	0.968	0.955	0.955	0.955	0.291	0.606	0.563	0.994	1	1	0.997	1	0.966	0.092	0.302	0.031
DQB1*03:02	0.855	0.911	0.933	0.951	1	1	1	0.993	0.974	1	1	1	1	1	1	0.931	0.870	0.500	0.067
DQB1*05:01	1	0.972	0.726	0.828	0.963	0.969	0.875	1	1	0.976	1	0.960	0.997	1	0.99	0.850	0.859	0.312	0.262
DQB1*06:02	0.959	0.979	0.994	1	1	1	0.991	1	1	1	1	0.995	1	1	1	0.828	0.739	0.678	0.594
DQB1*06:03	0.068	0.091	0.094	1	1	1	0.984	1	1	0.889	0.993	0.954	0.990	0.984	0.862	0.949	0.871	0.350	0.021
DPA1*01:03	0.076	0.250	0.070	0.177	0.312	0.208	0.260	0.100	0.011	0.205	0.154	0.185	0.031	0.281	0.232	0.381	0.126	0.065	0.551
DPA1*02:01	0.178	0.286	0.032	0.095	0.281	0.172	0.335	0.127	0.059	0.211	0.290	0.405	0.046	0.278	0.238	0.368	0.153	0.058	0.543
DPB1*02:01	0.108	0.015	0.032	0.005	0.020	0.024	0.046	0.080	0.205	0.051	0.132	0.058	0.066	0.031	0.021	0.045	0.429	0.029	0.027
DPB1*03:01	0.067	0.008	0.055	0.072	0.040	0.004	0.256	0.102	0.063	0.022	0.036	0.034	0.028	0.078	0.062	0.084	0.193	0.090	0.131
DPB1*04:01	0.015	0.003	0.071	0.026	0.068	0.107	0.161	0.024	0.046	0.116	0.028	0.098	0.005	0.060	0.051	0.112	0.059	0.048	0.138
DPB1*04:02	0.047	0.170	0.087	0.089	0.106	0.089	0.025	0.192	0.103	0.073	0.011	0.338	0.085	0.129	0.064	0.147	0.419	0	0.152

Table E3. LD (r^2) between 19 tag SNPs (selected according to GABRIEL asthma meta-analysis) and 23 HLA class II alleles. LD between HLA-DP alleles and six SNPs could not be computed (NA: not available).

r^2	rs3763309	rs9268542	rs2239804	rs13209234	rs34442410	rs28530648	rs9270856	rs9271488	rs3129763	rs35399661	rs9273349	rs9273448	rs9275141	rs5000634	rs1794275	rs3104405	rs9275602	rs241425	rs3132131
DRB1*01:01	0.02 6	0.07 2	0.10 5	0.62 5	0.86 8	0.74 7	0.29 0	0.04 4	0.03 5	0.52 3	0.14 7	0.02 9	0.10 7	0.06 6	0.44 1	0.18 9	0.01 0	0.02 2	0.04 4
DRB1*03:01	0.02 6	0.00 7	0.02 1	0.01 9	0.01 3	0.01 6	0.04 0	0.04 9	0.35 8	0.02 2	0.08 0	0.03 4	0.12 1	0.07 4	0.02 6	0.00 1	0.31 5	0.05 1	0.02 0.02
DRB1*04:01	0.33 2	0.11 9	0.08 1	0.01 4	0.00 9	0.01 2	0.03 0	0.21 2	0.02 9	0.01 7	0.06 2	0.02 6	0.08 3	0.13 5	0.02 0	0.03 2	0.02 9	0.02 5	0.00 4
DRB1*07:01	0 0	0.02 9	0.05 0	0.00 6	0.01 7	0.02 2	0.05 3	0.38 8	0.05 3	0.03 2	0.11 2	0.04 7	0.15 1	0.02 0	0.03 6	0.07 4	0.02 1	0.00 2	0.03 3
DRB1*15:01	0.03 0	0.08 7	0.13 9	0.02 2	0.01 5	0.01 8	0.40 3	0.05 6	0.04 5	0.02 7	0.19 4	0.37 0	0.10 8	0.08 0	0.02 9	0.16 5	0.01 0	0.07 1	0.09 4
DQA1*01:01	0.03 6	0.02 6	0.03 4	0.42 0	0.60 6	0.70 0	0.20 0	0.07 3	0.05 8	0.85 3	0.25 2	0.04 7	0.18 6	0.11 5	0.75 1	0.13 9	0.01 3	0.00 9	0.01 3
DQA1*01:02	0.04 9	0.14 6	0.13 0	0.02 5	0.02 2	0.02 6	0.28 9	0.09 2	0.07 3	0.03 9	0.31 6	0.50 0	0.12 7	0.06 5	0.04 8	0.20 3	0.01 6	0.06 1	0.03 5
DQA1*01:03	0.00 1	0 0	0.00 2	0.01 4	0.00 9	0.01 2	0.01 2	0.03 6	0.02 8	0.01 3	0.12 2	0.21 0	0.06 5	0.05 5	0.01 3	0.03 7	0.00 9	0.01 3	0 0
DQA1*02:01	0 0	0.02 9	0.05 0	0.00 7	0.01 7	0.02 2	0.05 3	0.38 9	0.05 3	0.03 2	0.11 2	0.04 7	0.15 2	0.02 0	0.03 6	0.07 4	0.02 1	0.00 2	0.03 2
DQA1*03:01	0.49 0	0.22 1	0.15 3	0.02 8	0.02 0	0.02 5	0.06 1	0.43 6	0.05 9	0.03 6	0.12 7	0.05 4	0.17 1	0.25 2	0.03 4	0.06 1	0.01 2	0.02 7	0.00 1
DQA1*05:01	0.03 4	0.06 7	0.01 0	0.05 5	0.03 6	0.04 6	0.11 2	0.14 7	0.92 6	0.03	0.24 4	0.10 4	0.01 2	0.06 5	0.07 9	0.06 6	0.10 0	0.05 7	0.00 5
DQB1*02:01	0.01 1	0.02 1	0.01 8	0.04 5	0.02 9	0.03 7	0.09 1	0.06 3	0.08 5	0.05 4	0.18 9	0.07 9	0 0	0.17 0	0.06 1	0.04 2	0.10 0	0.00 7	0.05 1

DQB1*03:01	0.01 3	0.21 6	0.09 6	0.03 9	0.02 5	0.03 1	0.07 8	0.00 9	0.28 2	0.01 6	0.17 5	0.07 5	0.23 9	0.38 6	0.05 7	0.10 9	0.00 5	0.01 7	0.00 1
DQB1*03:02	0.33 0	0.13 2	0.09 1	0.01 6	0.01 2	0.01 5	0.03 7	0.26	0.03 4	0.02 2	0.07 6	0.03 2	0.10 4	0.16 8	0.02 5	0.04 4	0.01 1	0.02 0	0
DQB1*05:01	0.03 3	0.08 8	0.07 5	0.56 2	0.79 2	0.91 3	0.30 7	0.05 6	0.04 4	0.65	0.19 3	0.03 7	0.14 2	0.08 8	0.58 9	0.21 0	0.01 4	0.01 8	0.02 0
DQB1*06:02	0.02 8	0.08 2	0.13 0	0.02 0	0.01 4	0.01 7	0.36 5	0.05 2	0.04 1	0.02 5	0.17 8	0.41 8	0.13 2	0.08 1	0.02 8	0.18 5	0.00 9	0.07 8	0.09 7
DQB1*06:03	0.00 1	0.00 1	0.00 1	0.01 3	0.00 9	0.01 1	0.02 6	0.03 2	0.02 6	0.01 2	0.11 1	0.24 1	0.08 2	0.05 0	0.01 3	0.03 3	0.00 8	0.01 3	0
DPA1*01:03	0	0.01 0	0.00 1	0.00 1	0.00 3	0.00 1	0.00 6	0.00 6	0	0.00 2	0.00 4	0.00 2	0	0.01 3	0.00 3	0.01 6	0.00 9	0.00 1	0.03 3
DPA1*02:01	0.00 1	0.01 1	0	0	0.00 2	0.00 1	0.00 7	0.00 7	0.00 2	0.00 2	0.01 1	0.00 9	0	0.00 9	0.00 2	0.01 2	0.01 7	0	0.02 5
DPB1*02:01	0.00 1	0	0	0	0	0	0	0	0.00 2	0.00 2	0.00 4	0.00 2	0.00 1	0	0	0	0.00 5	0	0
DPB1*03:01	0	0	0	0	0	0	0.00 2	0	0	0	0	0	0	0.00 1	0.00 2	0.00 2	0.00 1	0.00 1	0.00 1
DPB1*04:01	0	0	0.00 4	0	0	0.00 1	0.01 3	0	0	0.00 2	0.00 1	0.00 4	0	0.00 4	0	0.00 9	0.00 1	0.00 2	0.01 3
DPB1*04:02	0.00 1	0.00 5	0.00 1	0.00 6	0.01 0	0.00 7	0	0.00 2	0.00 4	0.00 3	0	0.00 4	0.00 1	0.00 3	0.00 2	0.00 1	0.00 3	0	0.00 6

Figure E1. Proportion of adult onset asthma among ESE subjects, according to carriage of the *DPB1*03:01* allele (- = 0 copies, + = 1 or 2 copies) and exposure to occupational HMW allergens (- = never, + = ever exposed).

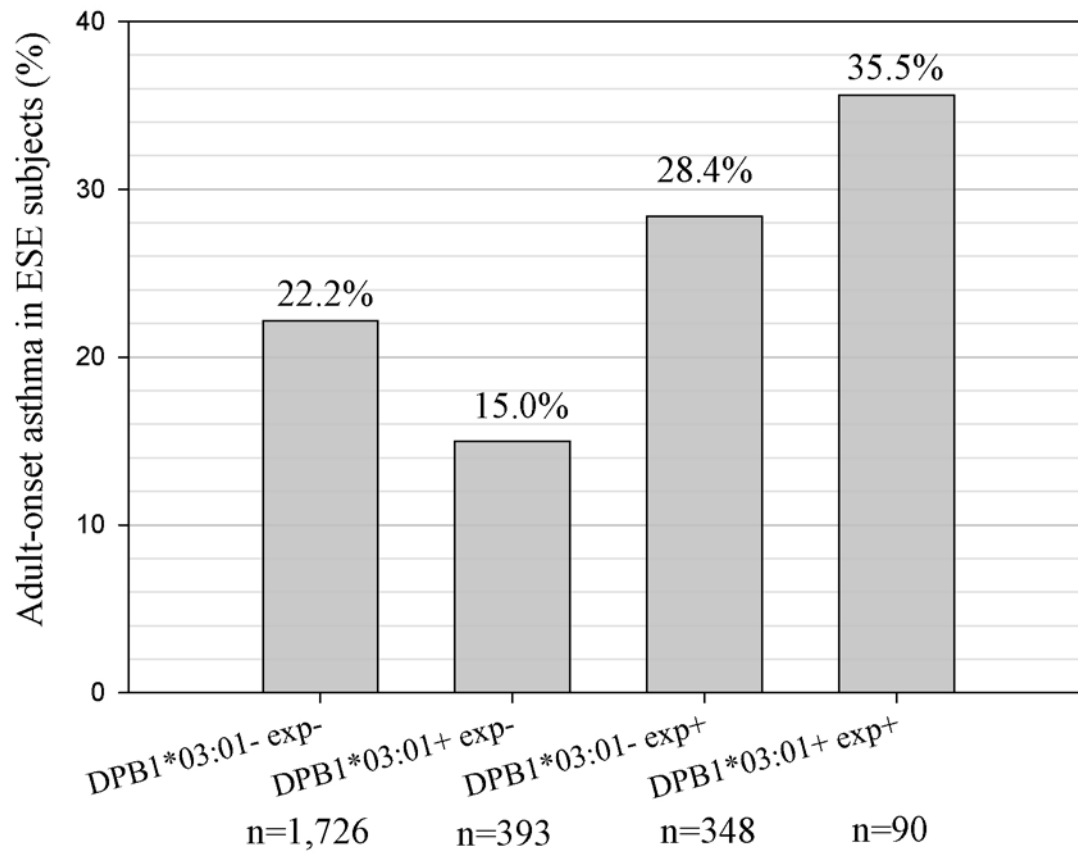


Table E4. Association of imputed classical HLA-II alleles and adult-onset asthma in subjects ever occupationally exposed to latex, and in subjects never exposed to HMW allergens. Results from fixed effects meta-analysis of ESE and B58C.

HLA-II allele	AF	HMW exposed, Latex (n=1,123) OR (95% CI)	Non-exposed (n=3,396) OR (95% CI)	p value joint test	p value interaction
DRB1*01:01	9.4	0.87 (0.56-1.36)	0.83 (0.66-1.04)	0.24	0.85
DRB1*03:01	12.6	1.02 (0.71-1.46)	1.11 (0.91-1.36)	0.55	0.69
DRB1*04:01	9.9	1.15 (0.78-1.70)	1.10 (0.87-1.38)	0.54	0.85
DRB1*07:01	13.3	1.24 (0.87-1.76)	1.01 (0.83-1.23)	0.47	0.33
DRB1*15:01	12.5	0.81 (0.55-1.21)	0.88 (0.72-1.07)	0.28	0.72
DQA1*01:01	15.1	0.89 (0.62-1.27)	0.88 (0.73-1.06)	0.37	0.98
DQA1*01:02	18.4	0.90 (0.65-1.25)	0.90 (0.76-1.06)	0.41	0.99
DQA1*01:03	6.8	0.84 (0.51-1.39)	0.83 (0.64-1.07)	0.31	0.96
DQA1*02:01	13.4	1.23 (0.87-1.75)	1.01 (0.83-1.23)	0.49	0.33
DQA1*03:01	18.6	1.08 (0.80-1.46)	1.11 (0.93-1.32)	0.41	0.87
DQA1*05:01	25.1	1.01 (0.76-1.33)	1.16 (1.00-1.34)	0.12	0.38
DQB1*02:01	17.8	1.08 (0.79-1.47)	1.05 (0.89-1.24)	0.74	0.88
DQB1*03:01	19.1	1.09 (0.80-1.49)	1.14 (0.97-1.33)	0.23	0.84
DQB1*03:02	10.6	1.10 (0.74-1.61)	1.04 (0.85-1.29)	0.80	0.83
DQB1*05:01	12.3	0.95 (0.64-1.41)	0.86 (0.70-1.06)	0.37	0.66
DQB1*06:02	12.0	0.82 (0.55-1.23)	0.89 (0.73-1.09)	0.35	0.72
DQB1*06:03	6.3	0.90 (0.54-1.49)	0.81 (0.62-1.06)	0.31	0.72
DPA1*01:03	18.8	0.94 (0.66-1.33)	0.95 (0.78-1.15)	0.84	0.98
DPA1*02:01	14.9	1.01 (0.70-1.47)	0.92 (0.75-1.12)	0.72	0.65
DPB1*02:01	13.4	1.16 (0.82-1.63)	1.25 (1.05-1.50)	0.03	0.68
DPB1*03:01	10.3	1.21 (0.82-1.80)	0.67 (0.53-0.86)	0.005	0.01
DPB1*04:01	42.4	1.06 (0.82-1.36)	1.03 (0.90-1.18)	0.79	0.85
DPB1*04:02	11.1	0.80 (0.53-1.21)	1.05 (0.84-1.30)	0.53	0.26
rs9273349 T	40.8	0.82 (0.61-1.10)	0.82 (0.71-0.95)	0.02	1.00

AF: weighted average allele frequency in ESE and B58C. Boldface indicates p<0.05.

Table E5. Association of imputed classical HLA-II alleles and adult-onset asthma according to ever having been occupationally exposed to HMW allergens other than latex. Results from fixed effects meta-analysis of ESE and B58C.

HLA-II allele	AF	HMW exposed, other than latex (n=560) OR (95% CI)	Non-exposed (n=3,396) OR (95% CI)	p value joint test	p value interaction
DRB1*01:01	9.4	0.73 (0.35-1.48)	0.83 (0.66-1.04)	0.20	0.73
DRB1*03:01	12.6	1.09 (0.67-1.78)	1.11 (0.91-1.36)	0.52	0.95
DRB1*04:01	9.9	1.10 (0.58-2.09)	1.10 (0.87-1.38)	0.67	0.99
DRB1*07:01	13.3	0.91 (0.54-1.53)	1.01 (0.83-1.23)	0.93	0.71
DRB1*15:01	12.5	0.99 (0.60-1.64)	0.88 (0.72-1.07)	0.47	0.66
DQA1*01:01	15.1	1.20 (0.70-2.06)	0.88 (0.73-1.06)	0.35	0.29
DQA1*01:02	18.4	1.01 (0.65-1.56)	0.90 (0.76-1.06)	0.48	0.62
DQA1*01:03	6.8	0.58 (0.25-1.33)	0.83 (0.64-1.07)	0.17	0.42
DQA1*02:01	13.4	0.91 (0.54-1.53)	1.01 (0.83-1.23)	0.94	0.73
DQA1*03:01	18.6	1.11 (0.70-1.76)	1.11 (0.93-1.32)	0.42	0.99
DQA1*05:01	25.1	1.06 (0.73-1.56)	1.16 (1.00-1.34)	0.12	0.69
DQB1*02:01	17.8	1.14 (0.74-1.75)	1.05 (0.89-1.24)	0.69	0.73
DQB1*03:01	19.1	1.01 (0.66-1.56)	1.14 (0.97-1.33)	0.27	0.63
DQB1*03:02	10.6	1.48 (0.87-2.53)	1.04 (0.85-1.29)	0.31	0.23
DQB1*05:01	12.3	1.00 (0.56-1.79)	0.86 (0.70-1.06)	0.38	0.63
DQB1*06:02	12.0	1.12 (0.68-1.86)	0.89 (0.73-1.09)	0.49	0.40
DQB1*06:03	6.3	0.58 (0.24-1.41)	0.81 (0.62-1.06)	0.16	0.48
DPA1*01:03	18.8	0.87 (0.55-1.39)	0.95 (0.78-1.15)	0.76	0.75
DPA1*02:01	14.9	1.14 (0.69-1.88)	0.92 (0.75-1.12)	0.63	0.42
DPB1*02:01	13.4	0.76 (0.42-1.37)	1.25 (1.05-1.50)	0.03	0.11
DPB1*03:01	10.3	0.80 (0.44-1.45)	0.67 (0.53-0.86)	0.006	0.60
DPB1*04:01	42.4	0.98 (0.69-1.41)	1.03 (0.90-1.18)	0.89	0.82
DPB1*04:02	11.1	1.22 (0.73-2.04)	1.05 (0.84-1.30)	0.67	0.60
rs9273349 T	40.8	0.81 (0.53-1.23)	0.82 (0.71-0.95)	0.02	0.94

AF: weighted average allele frequency in ESE and B58C. Boldface indicates p<0.05.

Table E6. Association of 19 tag SNPs in the HLA class II region and adult-onset asthma in the industrial cohorts of agricultural workers and bakery workers, all subjects ever exposed to occupational HMW allergens (industrial cohorts, ESE and B58C), and non-exposed subjects.

SNP	Position, (bp, build 36.3)	Baseline/ Effect Allele	AF	Industrial cohorts, ESE and B58C				
				Industrial only (n=946) OR (95% CI)	All HMW exposed (n=2,629) OR (95% CI)	Non-exposed (n=3,396) OR (95% CI)	p value joint test	p value interaction
rs3763309	32,483,951	A/C	79.3	0.83 (0.66-1.05)	0.86 (0.73-1.02)	0.90 (0.76-1.05)	0.11	0.75
rs9268542	32,492,699	A/G	46.9	1.16 (0.96-1.40)	1.15 (1.00-1.32)	1.18 (1.03-1.35)	0.006	0.76
rs2239804	32,519,501	C/T	49.6	0.88 (0.73-1.06)	0.90 (0.79-1.03)	0.81 (0.71-0.93)	0.004	0.27
rs13209234	32,523,953	A/G	86.0	1.63 (1.24-2.15)	1.30 (1.07-1.59)	1.08 (0.89-1.31)	0.02	0.18
rs34442410 ^a	32,550,064	C/G	11.1	0.54 (0.39-0.75)	0.61 (0.46-0.80)	0.84 (0.65-1.08)	0.001	0.10
rs28530648 ^a	32,635,057	A/C	13.1	0.56 (0.41-0.76)	0.64 (0.49-0.83)	0.85 (0.68-1.06)	0.001	0.11
rs9270856 ^a	32,678,817	A/G	74.2	1.06 (0.86-1.30)	1.12 (0.93-1.35)	1.09 (0.91-1.30)	0.26	0.82
rs9271488	32,696,978	G/T	30.8	1.06 (0.86-1.31)	1.08 (0.93-1.26)	1.07 (0.93-1.24)	0.32	0.94
rs3129763	32,698,903	A/G	75.9	0.89 (0.72-1.09)	0.92 (0.79-1.08)	0.87 (0.75-1.01)	0.13	0.62
rs35399661 ^a	32,698,968	C/T	83.9	1.71 (1.29-2.26)	1.41 (1.12-1.78)	1.08 (0.88-1.31)	0.01	0.08
rs9273349	32,733,847	C/T	41.6	0.89 (0.74-1.07)	0.86 (0.74-0.99)	0.82 (0.71-0.95)	0.005	0.69
rs9273448 ^a	32,735,725	A/G	76.4	0.89 (0.73-1.09)	0.99 (0.82-1.18)	1.18 (0.98-1.41)	0.19	0.18
rs9275141	32,759,095	G/T	49.8	0.90 (0.74-1.09)	0.84 (0.73-0.96)	0.87 (0.77-1.00)	0.009	0.68
rs5000634	32,771,542	A/G	39.0	1.12 (0.92-1.36)	1.07 (0.93-1.24)	1.16 (1.02-1.33)	0.04	0.42
rs1794275	32,779,226	A/G	82.7	1.56 (1.20-2.02)	1.25 (1.04-1.51)	1.09 (0.92-1.29)	0.03	0.29
rs3104405	32,790,286	A/C	33.1	0.96 (0.79-1.17)	0.94 (0.81-1.09)	0.89 (0.77-1.03)	0.23	0.58
rs9275602	32,790,790	A/C	84.7	0.92 (0.72-1.17)	0.95 (0.79-1.14)	0.99 (0.82-1.20)	0.87	0.74
rs241425	32,912,887	A/G	56.8	0.96 (0.80-1.16)	1.05 (0.91-1.20)	1.20 (1.06-1.37)	0.01	0.15
rs3132131	33,007,463	A/G	67.5	0.98 (0.80-1.19)	1.07 (0.92-1.23)	1.13 (0.98-1.30)	0.15	0.60

^aSNP not available in B58C dataset. AF: weighted average effect allele frequency in industrial cohorts, ESE and B58C. Boldface indicates p<0.05.