

Original article

HLA Dr-Dq haplotypes and the TNFA-308 polymorphism: associations with asthma and allergy

Background: The HLA (human leukocyte antigen) class II genes *DQB1* and *DRB1* and the Tumor Necrosis Factor α gene (*TNFA*) within the HLA complex (chromosome 6p21) have been associated with asthma and allergy. Due to the strong linkage disequilibrium characterizing this complex and the multiple asthma/allergy expressions, we aimed to determine which of these genes were primarily involved in specific asthma/allergy traits.

Methods: The *DRB1*–*DQB1* alleles and *TNFA*-308 polymorphism were genotyped in 959 children from the Environment and Childhood Asthma study and analyzed for possible associations with allergic and non-allergic asthma (with/without at least one positive skin prick test for allergens) and specific allergic sensitization, as well as bronchial hyperresponsiveness and total IgE, using both allele and extended haplotype analyses.

Results: Different genes within the HLA complex were associated with separate asthma and allergy traits. Nonallergic asthma was associated with both the *TNFA*-308A allele [Odds ratio (OR) 1.7 (1.3–2.3)] and *DRB1**03 allele [OR 1.6 (1–2.6)], but extended *DRB1**03–*TNFA*-308 haplotype analysis suggested that the *DRB1*–*DQB1* association was secondary to linkage disequilibrium with the *TNFA*-308 polymorphism. Allergies were associated with HLA class II alleles only; birch sensitization with *DQB1**0603–*DRB1**13 [OR 2.3 (1.4–4.0)] and mugwort sensitization with *DQB1**0609–*DRB1**13 [OR 7.1 (1.9–27.0)] and *DQB1**0501–*DRB1**01 [OR 2.0 (1.0–4.0)].

Conclusions: Our data suggests that asthma is not associated with *DRB1* or *DQB1* but rather *TNFA* or a gene(s) in linkage disequilibrium, while sensitization to specific allergens is associated with particular alleles at the DQ and/or DR loci. A novel association between *DQB1**0603–*DRB1**13 and birch allergy is identified.

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Asthma and allergies are common complex diseases with multiple determinants including genetic variation and gene–environment interactions (1). Asthma prevalence has increased world wide (2), with a recently demonstrated lifetime asthma prevalence of 20.2% in Norwegian urban school children (3). Major challenges include identifying groups genetically at risk for disease and developing tools for optimizing treatment.

The human leukocyte antigen (HLA) complex on chromosome 6p21 has been identified as one of the few chromosomal regions showing replicable linkage to asthma and allergy (4). The HLA class II genes *DRB1* and *DQB1*, and the Tumor Necrosis Factor α gene (*TNFA*) in the HLA complex cluster are among the 10

genes that have been replicated in at least 10 independent asthma studies (5). However, the genes in the HLA complex are in strong linkage disequilibrium (LD) with each other, and these LD patterns are frequently ethnicity specific, making it difficult to deduce which genes are primarily involved and which are only secondarily associated (6). Furthermore, although asthma and allergy are associated with each other with common genes contributing to disease disposition, it is also likely that specific aspects of asthma and allergy have separate underlying genetic architectures.

Both HLA-*DQB1* and HLA-*DRB1* are HLA class II genes that encode for the highly polymorphic β chain of HLA DQ and DR molecules, respectively (7). These molecules are cell surface glycoproteins with α and β chains forming heterodimers that present exogenous peptides (e.g. allergens) on antigen presenting cells (APC) to specifically matched T-cell receptors on T cells. Thus, configurations of the antigen-HLA molecule presented on APC's are important for stimulating specific T

Abbreviations: HLA, Human leukocyte antigen; TNF α , Tumor necrosis factor alpha; ECA, Environment and Childhood Asthma Study; FEV1, Forced expiratory volume in the first second; BHR, Bronchial Hyperresponsiveness; SPT, Skin Prick Test; SNP, Single Nucleotide Polymorphism; LD, Linkage disequilibrium.

cells and subsequent reactions. Several studies have reported association between different *DQB1* and *DRB1* alleles and allergic asthma (8–11) as well as with specific allergies (12–16), but the particular alleles involved have varied between studies.

The proinflammatory cytokine TNF α implicated in asthma (17) is usually associated with cell-mediated immunological responses. However, recent studies suggest that sensitized mast cells release preformed TNF α upon antigen presentation, that TNF α induces airway hyperreactivity, recruits neutrophils and eosinophils and activates myofibroblasts and fibroblasts (17), and that anti-TNF α treatment might be beneficial in patients with severe asthma (18). The *TNFA*-308 polymorphism has been extensively studied in relation to regulation of TNF α levels and asthma, but results have been inconsistent (19, 20).

The main aim of the present study was to assess whether *DQB1*, *DRB1* or *TNFA* were associated with subgroups of asthma or specific allergies in Norwegian children. Secondly, we sought to deduce which genes were primarily involved through comparisons of their extended *DQB1*–*DRB1*–*TNFA*-308 haplotype associations.

Subjects and Methods

Study design

The present study is a 10-year follow-up of children in the prospective birth cohort 'Environment and Childhood Asthma' (ECA) study in Oslo, described elsewhere (21). Briefly, a cohort of 3754 children was established at birth in Oslo during 15 months from January 1.1992. Written informed consent was obtained from parents of all subjects, and the study was approved by the regional medical ethics committee and the Norwegian Data Inspectorate.

The ECA is a prospective study that has to date contained two main phases. The initial study covered the first 2 years of life with questionnaires, lung function measurements at birth in 802 children, and a nested case-control study at 2 years including children with physician confirmed bronchial obstruction ($n = 306$) and age-matched controls ($n = 306$) whom underwent detailed examinations (21). The second phase was the 10-year follow-up study (2001–2004) including 1215 children with lung function measurements at birth and/or a clinical 2-year investigation, where 1019 children (84 percent) attended the investigations (3). The investigations included detailed parental structured interview, blood tests, skin prick tests for allergic sensitization, lung function by forced flow-volume measurements, methacholine challenge test, exhaled nitric oxide measurement, urine sampling and a clinical examination on day one, and an exercise test by treadmill running within 1 week. Blood for DNA analysis was sampled in 959 (94 percent) of the attending children.

Subjects

The present study comprises the 959 children with available DNA for analysis. Technical problems precluded DNA analysis in 13 subjects, and a further 48 subjects were excluded as one or both of their parents had other than Caucasian origin. In the remaining 898 subjects, the phenotypes were ascertained in the following number of subjects: asthma status; 898 subjects, allergic sensitization; 891

Table 1. Clinical baseline characteristics for the study population and the excluded subjects

Demographic information	Subjects included in the analysis		Subjects excluded from the analysis	
	<i>n</i>	(%)	<i>n</i>	(%)
Total ECA-2 subjects ($n = 1019$)	898	88	121	12
Gender male	484	54	65	55
Age at examination				
8–9	1	<1	0	0
9–10	176	20	25	21
10–11	387	43	50	42
11–12	278	31	31	26
12–13	56	6	13	11
Asthma	268	30	35	30
Nonallergic asthma	165	19	19	16
Allergic asthma	101	11	16	13
Parental asthma	163	18	15	13
Parental rhinoconjunctivitis	367	41	50	42
Allergic sensitization				
At least one positive SPT	254	28	39	32
Specific allergies by SPT				
Cat dander	100	11	20	17
Dog dander	76	9	11	9
Grass	138	15	18	15
Birch	128	14	23	19
Mugwort	48	5	4	3
Peanut	75	8	14	12
PD20 $\leq 8\mu\text{mol}$	278	31	47	39
Total IgE kU/l geo mean (SD) *	51.94 (3.67)	68.57 (3.28)		

*Total IgE is given as geometric mean (geo mean) with standard deviation (SD)

subjects, total IgE; 884 subjects, and BHR; 877 subjects. Comparisons between included and excluded subjects are given in Table 1.

Methods (at 10-year follow-up study)

Parental structured interview included central International Study of Asthma and Allergies in Children (ISAAC) questions related to airway symptoms [validated in Norwegian language (22)] of the child. The two clinical examinations were performed 2–7 days apart, at least 4 weeks after any suspicion of respiratory tract infection (3).

Forced expiratory flow volume loops were measured according to European standard (23) with the reference values of Zapletal (24) on a SensorMedics Vmax 20c (SensorMedics Diagnostics, Yorba Linda, CA, USA). The reported values were the best baseline values obtained before challenge testing (3).

Skin prick tests (SPT) were performed with the following prevalent food and inhalant allergens: Domestic mites [*Dermatophagoides pteronyssinus* (D.pter) and *D. farinae* (D.far)], German cockroach, dog, cat, and rabbit dander, birch, timothy (grass) and mugwort (*Artemisia vulgaris*) pollens, moulds (*Cladosporium herbarium* and *Alternaria*), egg white, milk, peanut and codfish (Soluprick[®], ALK Abello, Denmark). The SPT was considered positive with a wheal diameter to the particular allergen ≥ 3 mm larger than the negative control (NaCl) (25). Of all the allergens, only those with positive SPT frequencies exceeding five percent in the study population were included for further analysis (dog, cat, birch, timothy, mugwort and peanut).

Serum was analyzed for total immunoglobulin E (IgE) using the UniCAP fluoroenzyme immunoassay (FEIA) according to the manufacturer's instructions (Pharmacia Upjohn, Uppsala, Sweden).

Bronchial hyperresponsiveness (BHR) was measured by PD20 metacholine (26) according to international guidelines (27), and reported as the dose of metacholine that caused a fall in FEV1 of 20% from the (postsaline) baseline value.

Outcomes:

- 1 *Asthma ever* was defined by at least two of the following three criteria being fulfilled (3):
 - i Dyspnoea, chest tightness and/or wheezing within the first 10 years of life
 - ii Doctor's diagnosis of asthma
 - iii Use of asthma medication (β -2 agonist, sodium chromoglycate, corticosteroids, leukotriene antagonists and/or aminophylline) within the first 10 years of life
- 2 *Allergic sensitization* was defined as at least one positive SPT. Specific allergic sensitization was defined by having a specific positive SPT.
- 3 *Allergic asthma* was defined as asthma in the presence of allergic sensitization, and *non-allergic asthma* was defined as asthma without the presence of allergic sensitization.
- 4 *Total IgE* was log transformed to satisfy normal distribution and reported using geometric mean.
- 5 *BHR* was reported as a dichotomous variable with BHR present if PD20 methacholine was below 8 μ mol.

HLA class II typing and TNF genotyping

HLA *DRB1* genotyping was performed using the Dynal RELITMSSO kit (Invitrogen, Carlsbad, CA, USA). HLA *DQB1* genotyping was mainly performed using the Delfia kit (Perkin Elmer Life Sciences, Waltham, MA, USA) based on time-resolved fluorometry and the use of lanthanide labels. In cases of ambiguity, sequence-based typing of HLA-DQB1 was performed in addition by amplification of exon 2, followed by sequencing using BigDye[®] Terminator v3.1 chemistry (Applied Biosystems, Foster City, CA, USA) on an ABI3730 DNA Sequencer (Applied Biosystems) with allele assignment in AssignSBT[®] v3.2.7b (Conexio Genomics, Applecross, Australia) (28). Primer sequences and PCR conditions are available from the authors upon request. The TNFA-308 polymorphism was genotyped using the TaqMan[®] SNP genotyping assay c_7514879_10 (Applied Biosystems), and analyzed on an ABI 7900 HT Realtime PCR (Applied Biosystems).

Statistical analyses

Demographic data are given as count (%) and compared between groups using the Pearson Chi-Squared Test (Statistical Package for Social Sciences [SPSS] version 14; SPSS Inc., Chicago, IL, USA) unless otherwise stated.

Dichotomous variables were analyzed against *DQB1* and *DRB1* separately and as haplotypes (where the *DQB1-DRB1* haplotypes were deduced based upon known patterns of LD in the Norwegian population). Alleles and haplotypes with frequencies less than five percent in both asthma cases and controls were grouped into a common 'rare' category. The distribution of the alleles/haplotypes were scored for asthma categories, BHR and SPT positive groups (allergic groups) separately, and compared with the group of subjects without asthma, BHR or allergies, respectively. Due to the large number of *DQB1-DRB1* alleles and haplotypes several cells

had expected counts of less than five, for which reason a Monte Carlo test with 10 000 reiterations (SPSS 14) was applied in order to evaluate where the allele/haplotype distribution was significantly different for the two groups compared. The quantitative variable total IgE was log transformed and analyzed against the HLA alleles using the ANOVA test (SPSS 14). The global *P*-value for each analysis obtained and reported in the present study has been corrected according to Bonferroni and multiplied by the number of phenotypes analyzed (11 in the present study) minus one.

For traits with Bonferroni corrected global *P*-values below 0.05, multi-allelic regression analysis (a regression analysis where the factor covariates correspond to alleles or haplotypes) was performed to give risk estimates for individual alleles or haplotypes, and the reference was defined as the allele/haplotype most equally distributed and present in more than 10% of both case and control groups.

The TNF α -308 alleles (A and G) were analyzed for associations with dichotomous variables using Pearson chi-squared test and quantitative variables using ANOVA, and interpreted using a Bonferroni corrected significance level of 0.05.

Results

Among all included subjects [mean age 10.7 years (SD 0.8), 54% boys], 30% had 'asthma ever', 28% were allergically sensitized and 31% exhibited BHR. Among the children with 'asthma ever' (*n* = 268), 38% were sensitized to at least one allergen and categorized as allergic asthma, while 62% were SPT negative and categorized as nonallergic asthma. The included subjects did not differ significantly from the excluded subjects with regard to asthma or allergy (Table 1).

Global association tests

The primary analysis where allele distributions were tested for differences between the asthma/allergy phenotypes and control groups identified the following Bonferroni corrected significant associations (Table 2):

'Asthma ever' and nonallergic asthma were associated with *DRB1* allele distribution (*P* = 0.006 and 0.02 respectively), as well as *TNFA* allele distribution (*P* = 0.003 and *P* = 0.001 respectively).

Allergic sensitization (at least one positive SPT) was not associated with either HLA class II genes or *TNFA*. However, significant associations were found between specific sensitization to cat dander and *DQB1* allele distribution (*P* = 0.02), birch sensitization and *DQB1* allele distribution (*P* = 0.0006) and *DQB1-DRB1* haplotype distribution (*P* = 0.02), while mugwort was associated with *DQB1-DRB1* haplotype distribution (*P* = 0.003).

Bronchial hyperresponsiveness and IgE levels were not associated with either HLA class II alleles or *TNFA*.

Multi-allelic regression analysis

The multi-allelic regression analysis was only performed for clinical categories where the Bonferroni corrected *P*-value was below 0.05.

Table 2. Distribution of DQB1–DRB1 alleles and haplotypes and TNFA-308 alleles within the 12 tested clinical categories

Asthma/allergy categories	DQB1 distribution	DRB1 distribution	DQB1–DRB1 distribution	TNFA
1. Asthma ever				
p*	0.006	0.0005	0.04	0.0003
pc**	0.07	0.006	0.44	0.003
2. Allergic asthma				
p	0.02	0.02	0.09	0.11
pc	0.22	0.22	0.99	>1
3. Non-allergic asthma				
p	0.01	0.002	0.12	0.0001
pc	0.11	0.02	>1	0.001
4. Total IgE (log)				
p	0.08	0.09	0.23	0.12
pc	0.88	0.99	>1	>1
5. PD20 ≤ 8 μmol				
p	0.15	0.29	0.15	0.13
Pc	>1	>1	>1	>1
6. Allergic sensitization				
p	0.006	0.43	0.02	1.0
pc	0.07	>1	0.22	>1
7. SPT cat				
p	0.002	0.05	0.04	0.12
pc	0.02	0.55	0.44	>1
8. SPT dog				
p	0.02	0.06	0.18	0.61
pc	0.22	0.66	>1	>1
9. SPT grass				
p	0.02	0.06	0.15	0.16
pc	0.22	0.66	>1	>1
10. SPT birch				
p	0.00005	0.05	0.002	0.74
pc	0.0006	0.55	0.02	>1
11. SPT mugwort				
p	0.01	0.02	0.0003	0.06
pc	0.11	0.22	0.003	0.66
12. SPT peanut				
p	0.04	0.21	0.09	0.49
pc	0.44	>1	0.99	>1

The table depicts the global *P* values for differences in allele and haplotype distribution between cases and controls. Significant *P*-values after Bonferroni correction are in boldface.

SPT, Skin prick test.

p* uncorrected *P*-value.

pc** Bonferroni corrected *P*-value.

Asthma. Non-allergic asthma was associated with *DRB1**03 [odds ratio (OR) (95% confidence interval) 1.6 (1.0–2.6)] *P* = 0.04 (Table 3a), but ‘asthma ever’ did not demonstrate any specific *DRB1* allele association. The *TNFA*-308 analysis demonstrated association with the A allele for both categories, with OR 1.6 (1.2–2.0) for ‘asthma ever’, and OR 1.7 (1.3–2.3) for non-allergic asthma (Table 4).

Specific sensitization. Cat dander sensitization was not associated with any specific *DQB1* allele, whereas birch sensitization was associated with the *DQB1**0603 allele (OR 2.2 (1.3–3.6) *P* = 0.002) and the *DQB1**0603-*DRB1**13 haplotype (OR 2.3 (1.4–4.0) *P* = 0.002)

(Table 3b and c). Mugwort sensitization was associated with the HLA haplotypes *DQB1**0609-*DRB1**13 (OR 7.1 (1.9–27.0), *P* = 0.004) and *DQB1**0501-*DRB1**01 (OR 2.0 (1.0–4.0), *P* = 0.04) (Table 3c).

Extended haplotype test

As non-allergic asthma was associated with both *DRB1**03 and *TNFA*-308 A, and a strong LD was found between these two alleles (85% of *DRB1**03 alleles occurred on the same haplotype as *TNFA*-308A and 66% of *TNFA*-308A occurred with *DRB1**03), analysis of the extended haplotypes based on these two alleles was performed (Fig. 1). Individual extended haplotype risk analyses demonstrated that both the extended haplotypes including *TNFA*-308A were overrepresented among non-allergic asthma subjects, albeit only reaching statistical significance when together with *DRB1**03.

Discussion

The present study suggests that several genes within the HLA complex on chromosome 6p21 may be associated with specific asthma and allergy traits. The *TNFα* gene was associated with nonallergic asthma in children, while specific HLA class II *DQB1* and *DRB1* alleles were associated with specific sensitization.

The association between genes within the HLA complex and asthma and allergy are supported by several large genome-wide linkage studies demonstrating involvement of the HLA complex in the development of asthma or allergies (4). It has recently become apparent that many of the regions showing consistent linkage to asthma or allergy in multiple studies harbor more than one susceptibility locus with smaller individual effects (as opposed to single genes with large effects), such as has been demonstrated for the linkage to 5q31-33 (5). The present study is thus in line with evidence of multiple susceptibility loci within 6p21-22, and furthermore suggests that these loci affect different aspects of the allergy and asthma phenotypes. Similar findings of disease related phenotypes and multiple susceptibility loci are reported for several autoimmune diseases (6).

In addition to linkage studies, our results are supported by several genetic association studies reporting association between allergic asthma and the HLA class II alleles [*DQB1**0301-*DRB1**11 (9), *DRB1**03 (11, 29), *DQB1**0501-*DRB1**01 (10) and *DRB1**04 (8)] and the *TNFA*-308 polymorphism (19), as well as association between particular HLA class II alleles and specific allergies such as mite allergy (13, 14), cockroach allergy (15) and peanut allergy (12). However, faced with such a plethora of associations and phenotypes, two fundamental issues are addressed in order to interpret our results.

First, given the strong LD between *TNFA* and HLA class II alleles demonstrated in the present study as well

Table 3. Multi-allelic regression analysis of specific HLA class II alleles and haplotypes for the associated categories

DRB1 alleles	No asthma	Non-allergic asthma	
	<i>n</i> (%)	<i>n</i> (%)	OR (95% CI)
(a) DRB1 alleles and association with non-allergic asthma			
01	140 (11)	36 (11)	Reference
03	147 (12)	62 (19)	1.6 (1.0–2.6) *
04	286 (23)	74 (22)	1.0 (0.6–1.6)
07	112 (9)	34 (10)	1.2 (0.7–2.0)
11	44 (4)	19 (6)	1.7 (0.9–3.2)
13	163 (13)	40 (12)	1.0 (0.6–1.6)
15	219 (17)	38 (12)	0.7 (0.4–1.1)
Rare	149 (12)	27 (8)	0.7 (0.4–1.2)
Total	1260	330	
(b) DQB1 alleles and association with birch sensitization			
DQB1 Alleles	No allergy	Birch sensitization	
	<i>n</i> (%)	<i>n</i> (%)	OR (95% CI)
02	258 (20)	54 (21)	Reference
0301	165 (13)	30 (12)	0.9 (0.5–1.4)
0302	203 (16)	34 (13)	0.8 (0.5–1.3)
0501	151 (12)	19 (8)	0.6 (0.3–1.1)
0602	203 (16)	52 (20)	1.2 (0.8–1.9)
0603	81 (6)	37(15)	2.2 (1.3–3.6) **
Rare	213 (17)	29 (11)	0.7 (0.4–1.1)
Total	1274	255	
(c) DQB1–DRB1 haplotypes and association with birch and mugwort sensitization			
DQB1–DRB1 Haplotypes	No allergy	Birch	Mugwort
	<i>n</i> (%)	<i>n</i> (%)	OR (95% CI)
02–03	179 (14)	36 (14)	Reference
02–07	78 (6)	17 (7)	1.1 (0.5–2.0)
0301–04	75 (6)	11 (4)	0.7 (0.4–1.5)
0301–11/12/13	87 (7)	18 (7)	1.0 (0.6–1.9)
030201–04	202 (16)	34 (13)	0.8 (0.5–1.4)
0303–07	42 (3)	7 (3)	0.8 (0.3–2.0)
0303–09	14 (1)	2 (1)	0.7 (0.2–3.3)
04–08	47 (4)	5 (2)	0.5 (0.2–1.4)
0501–01	135 (11)	18 (7)	0.7 (0.4–1.2)
0501–10	15 (1)	1 (<1)	0.3 (0.1–2.6)
0502–16	7 (1)	0	0 (0- > 100)
0503–14	27 (2)	5 (2)	0.9 (0.3–2.6)
0601–15	6 (1)	0	0 (0- > 100)
0602–15	200 (16)	52 (20)	1.3 (0.8–2.1)
0603–13	77 (6)	36 (14)**	2.3 (1.4–4.0)
0604–13	64 (5)	6 (2)	0.4 (0.2–1.1)
0609–13	5 (<1)	3 (1)	2.8 (0.8–10.2)
Rare	14 (1)	4 (2)	1.4 (0.4–4.6)
Total	1274	255	96

n (%) refers to number of haplotype alleles and percentage within each phenotype.

OR (95%CI), Odds ratio with 95% confidence interval. Values in bold indicate $P < 0.05$.

* $P < 0.05$; ** $P < 0.005$.

as in earlier studies (30), it is advantageous to analyze the two genes in relationship to each other for possible asthma associations. Based on our extended haplotype analysis, the present study suggested that the association between *DRB1**03 and asthma was largely secondary to LD with the *TNFA*-308A allele. Consequently asthma as such was not directly influenced by alleles of the DR or DQ loci, but rather by the *TNFA*-308A allele or another allele in LD with *TNFA*-308A. Two previous studies (11,

31) evaluating *TNFA* and HLA class II alleles support the present findings of an association between the *DRB1**03-*TNFA*-308A haplotype and asthma. However, while the Australian study (31) concluded that the effect on asthma was a haplotype effect (and the etiological gene not identified), the Italian study (11) suggested that the two alleles additively predispose towards allergic asthma. The latter could not be confirmed in the present study as the remaining non-*DRB1**03- *TNFA*-308A alleles were

Table 4. Distribution of TNF α -308 alleles within the asthmatic and allergic phenotypes

Clinical trait	TNF α -308 A allele		
	n (% with trait)	n (% without)	Odds ratio (95% CI)
Asthma ever	130 (24)	213 (17)	1.6 (1.2–2.0)
Allergic asthma	43 (22)	213 (17)	1.3 (0.9–2.0)
Nonallergic asthma	87 (26)	213 (17)	1.7 (1.3–2.3)

n = number of alleles

underrepresented in the specific allergic asthma groups (data not shown). Consequently, the present study is in line with the hypothesis that HLA class II molecules are not involved in the asthma etiology. However, the role of *TNFA* has also been subject to conflicting *TNFA*-308-asthma association results. This may be due to *TNFA* being in LD with yet another etiological asthma gene, where the conflicting associations are caused by varying LD patterns in different populations. On the other hand, *TNFA*-308 study samples may have been inadequately powered, as the effect of *TNFA* on asthma is relatively small (OR 1.7) and previous meta-studies conclude with a *TNFA*-308 A allele effect (20, 32).

Secondly, in addition to the association between *TNFA* and nonallergic asthma, the present study also detected associations between specific birch and mugwort sensitization and particular HLA class II alleles. As there was no association between total IgE levels or general allergic sensitization and *DQB1*–*DRB1* alleles, we suggest that these associations are allergen specific. This is in line with a hypothesis that presentation of allergens in the context of HLA class II molecules by APCs to CD4 + T cells is important in the allergic response. These phenotype-specific associations further support the notion that the genetic architecture underlying specific allergies and asthma may be different from each other. Most of the previous HLA class II-asthma association studies that

have detected association with allergic asthma (8–10) did not further subcategorize the phenotype. Given the many different *DQB1*–*DRB1* allele associations reported, it is possible that these may primarily represent associations with co-existing allergy and not asthma *per se*, giving rise to a potentially large number of different haplotype associations depending on the prevalence of specific allergies in the populations studied.

Birch allergy is common and typical for the Scandinavian countries, and to our knowledge associations between birch allergen and specific *DQB1*–*DRB1* haplotypes have not previously been reported. Although the present study detected association between *DQB1**0603 and birch sensitization alone, we cannot conclude that the *DQB1* allele is responsible for the association. Due to the strong LD between particular alleles at the DR and DQ loci it is difficult to distinguish individual allelic effects. Mugwort sensitization (less common in our study population) was associated with *DQB1**0609–*DRB1**13 (a rare haplotype in Caucasian populations) and *DQB1**0501–*DRB1**01, supported by a study demonstrating association between this allele and mugwort induced asthma (10) as well as an Austrian study demonstrating significantly higher expression of *DRB1**01 molecules in patients with mugwort allergy (16). Detecting such specific HLA class II allele-allergy associations have been suggested as a possible basis for exploring new immunotherapeutic approaches (16).

In conclusion, the present study suggests that different genes within the HLA complex are associated with separate asthma and allergy traits. Our data suggests that the linkage and association between the HLA complex and asthma and allergy may in part be due to an association between the TNF α gene (or a gene in LD with *TNFA*) and non-allergic asthma, as well as HLA class II allele involvement in specific allergic responses. As such, a novel association between *DQB1**0603–*DRB1**13 and birch allergy was identified.

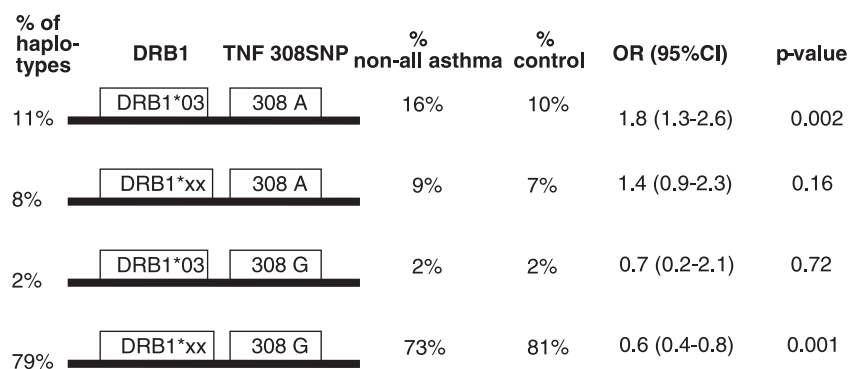


Figure 1. Extended *DRB1**03–TNF α -308 haplotypes among subjects and associations with non-allergic asthma. Global *P* value = 0.002 *DRB1**xx denotes all the non *DRB1**03 alleles. The figure illustrates the four possible haplotypes based on the presence or absence of the *DRB1**03 allele and the TNF α 308 polymorphism, and how these are distributed among the subjects. The columns to the right indicate the associations between single haplotypes and asthma based on odds ratios and uncorrected *P*-values for individual haplotype risk estimates.

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