

Association of HLA-DQ5 and HLA-DR1 with sensitization to organic acid anhydrides

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Summary

Background Organic acid anhydrides are low molecular weight industrial chemicals, able to cause rhinoconjunctivitis and asthma associated with specific IgE against hapten–carrier protein conjugate. Only a proportion of exposed workers develop IgE-associated allergy to acid anhydrides.

Objective We determined whether genetic susceptibility, in particular, HLA Class II alleles may be a risk factor.

Methods We undertook HLA typing in 52 cases who had confirmed specific IgE and in 73 referents matched on site, age and duration of acid anhydride exposure identified in cross-sectional studies of workers exposed to hexahydrophthalic (HHPA), methylhexahydrophthalic (MHHPA) and methyltetrahydrophthalic (MTHPA) anhydrides.

Results The linked alleles DQ5 (odds ratio [OR] = 4.3; 95% confidence interval [95% CI] = 1.7, 11) and DR1 (OR 3.0; 95% CI 1.2, 11) were more prevalent in cases than in referents. Within DQ5, DQB1*0501 was particularly frequent (OR 3.0; 95% CI 1.2, 7.4).

Conclusion DQB1*05 gene confers susceptibility to develop specific IgE antibodies against HHPA, MHHPA and a non-significant trend with MTHPA. DQB1*0501 is protective for other low molecular chemical sensitizers (isocyanates and plicatic acid) which may indicate varying affinities for the corresponding specific class II molecules.

Keywords acid anhydrides, HLA class II, occupational allergy and asthma

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Introduction

We have investigated the relationship between HLA class II alleles and the development of specific IgE to acid anhydrides conjugated with human serum albumin. Acid anhydrides have been widely used in industrial processes for some 50 years. They are low molecular weight reactive chemicals used as intermediates in the manufacture of plasticizers, alkyd, epoxy and polyester resins, as well as in the manufacture of dyes, insecticides, pharmaceuticals, lubricating oil additives and paper [1]. Acid anhydrides are a well-recognized cause of occupational rhinoconjunctivitis and asthma, which is usually associated with specific IgE antibodies.

The development of specific IgE antibodies is likely to be determined by the interaction of both multiple genes and environmental factors. Sensitization in workers exposed to low molecular weight haptens provides the opportunity to study gene–environment interaction because the specific

immunological response to a single chemical hapten, which probably contains a single T cell epitope for presentation by class II alleles, can be studied within a defined work force with well-characterized exposure.

Associations between sensitization to low molecular weight chemicals and HLA class II alleles have been reported, although to date, few other studies have further tested these associations. Sensitization to trimellitic anhydride (TMA) and probably tetrachlorophthalic anhydride (TCPA) was associated with DR3; however, no association was observed with phthalic anhydride (PA) [2]. Different acid anhydrides vary in their structure, ring structure, position of double bonds and methyl group substitutions which will influence the binding of the anhydride to the highly polymorphic class II molecules. In view of these differences, it is of interest to study HLA associations with other acid anhydrides of varying chemical structure.

The aim of this case referent study was to investigate whether sensitization to the acid anhydrides, hexahydrophthalic anhydride (HHPA), methylhexahydrophthalic anhydride (MHHPA) and methyltetrahydrophthalic anhydride (MTHPA) was associated with particular DR and DQ alleles, and to determine whether the risk of occupational sensitization to acid anhydrides varied in genetically suscep-

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tible individuals at different levels of exposure; in particular, whether the strength of any association was greater in those who had experienced lower levels of exposure to the anhydrides.

Methods

Factories

We studied employees from two factories in Sweden. One factory was situated in the south of Sweden (site 1) and manufactured electrical capacitors insulated by epoxy resin using HHPA or MHHPA as hardeners. The other factory was in the north of Sweden (site 2) and manufactured various plastic products, such as barrels for rocket guns, based on an epoxy resin system with MTHPA. This particular workforce from both factories have been extensively studied for sensitization to anhydrides, exposure levels, work related symptoms, atopic history and smoking habits [3, 4]. Atopic history was defined as a history of hayfever, asthma, atopic eczema or urticaria (when a history to a particular agent was convincing) during childhood or adolescence. Subjects were skin prick tested with 13 common allergens (Allergologisk, Copenhagen, Denmark) and considered atopic when one or more skin prick test was positive. Specific nasal challenges were performed in workers. Sensitized workers had nasal symptoms on exposure [5, 6].

Subjects

Recruitment of cases and referents was based on earlier cross-sectional studies of the entire working populations of the factories, which had been very complete [3, 4]. Cases were defined as workers with $>1.5\%$ specific IgE binding on RAST to at least one anhydride conjugated with human serum albumin [7, 8], at the time the blood for DNA analysis was taken. Referents were defined as not having specific IgE to any human serum albumin conjugate of acid anhydrides. One hundred and six cases and 162 referents were identified for the study, and blood was obtained from 89 cases and 135 referents. DNA was extracted for 74 (70%) of cases and 116 (72%) of referents. We undertook two studies, namely an overall case-control study and a matched case-control study. In the overall case-control study, HLA-DR and -DQ typing was undertaken in 74 cases and 116 referents. In the matched case-control study, we aimed to match one case to two referents. However, we were unable to match all cases and referents in this way. Fifty-two cases were matched on site and duration of exposure to acid anhydrides to 73 referents, 31 cases were matched to one referent and 21 cases were matched to two referents. Twenty-two cases had no matched referents and 10 potential referents had no matched cases because DNA could not be extracted. Cases and referents were similar in age, sex, smoking and intensity of exposure (Table 1). More cases than referents reported an atopic history (23% and 12%, respectively). However, this was not statistically significant $P=0.22$. Eight cases (15%) had asthma like symptoms compared with five referents (7%) $P=0.09$; 41 cases (79%) had rhinitis compared with 34 referents (47%) $P<0.001$.

Table 1. Characteristics of study groups

	Cases (<i>n</i> = 52)	Referents (<i>n</i> = 73)
Age: median (range) years	38 (26–72)	39 (26–70)
Sex: males <i>n</i> (%)	37 (71)	49 (67)
Current smokers: <i>n</i> (%)		
No	21 (40)	31 (43)
Yes	18 (35)	34 (47)
Ex-smoker	13 (25)	8 (11)
Atopic history: <i>n</i> (%)	12 (23)	9 (12)
Duration of exposure: mean (SD) years	16.4 (7.8)	15.0 (7.4)
Exposure: <i>n</i> (%)		
Low	19 (37)	29 (40)
Medium	16 (31)	21 (29)
High	17 (33)	23 (32)

Exposure levels

Workers were categorized into high-, medium- or low-exposure levels, according to the highest ever exposure levels of acid anhydride in their work history [3, 4]. Employees working in areas where the exposure level of acid anhydrides exceeded $50\mu\text{g}/\text{m}^3$ were categorized in the high group, those exposed to levels $10\text{--}50\mu\text{g}/\text{m}^3$ in the medium group and those exposed to less than $10\mu\text{g}/\text{m}^3$ in the low group. Average or cumulative exposure levels were not available for the cases and referents.

Genomic DNA preparation

Genomic DNA was extracted using a high-salt technique with minor modifications as previously described [9, 10].

Polymerase chain reaction-sequence-specific primer (PCR-SSP) analysis

HLA typing was done without knowledge of the subject's case status. HLA-DRB1 and -DQB1 were typed using the polymerase chain reaction with sequence-specific primers as previously described [11]. All PCR reactions were performed in a final reaction volume of $13\mu\text{L}$ excluding $10\mu\text{L}$ of light mineral oil. The PCR reaction mixture contained 67 mM Tris base pH 8.8, 16.6 mM NH_4SO_4 , 2 mM MgCl_2 , 0.01% (vol/vol) Tween 20, 200 μM of each deoxyribonucleoside triphosphate (dNTP) (deoxyadenosine, deoxyguanosine, deoxyadenosine, and deoxythymidine triphosphate), 1–4 μM of each allele-specific primer, 0.1 μM of each control primer (DRB exon 3, DRB exon 4), between 0.1 and 0.001 μg DNA, and 0.1875 U Taq DNA polymerase [11]. The sequence of addition of buffer mixture to specific primers has previously been described [11]. PCR amplifications were carried out in an MJ Research PTC-192 V machine (Genetic Research Instrumentation Ltd, Dunmow, Essex, UK).

The cycling parameters were as follows: 1 min at 96°C ; 5 cycles of 25 s at 96°C , 45 s at 72°C ; 21 cycles of 25 s at 96°C , 50 s at 65°C , 45 s at 72°C ; 4 cycles of 25 s at 96°C , 60 s at 55°C , 120 s at 72°C . The plates were sealed and the lid of the thermocycler was adjusted to ensure firm and even pressure on the plate within the machine.

The PCR products were electrophoresed (200 V, 15 min) in 1% agarose gels containing 0.5 µg/mL ethidium bromide after the addition of 10 µL of Orange G loading buffer to each well of the PCR plate and the results photographed.

Sub-typing of HLA-DQ5 was carried out as above using the following primers (MWG-Biotech AG):

DQB1*0501	5-ACG GAG CGC GTG CGG GG-3 5-GCT GTT CCA GTA CTC GGC AA-3
DQB1*0502	5-TGC GGG GTG TGA CCA GAC-3 5-TGT TCC AGT ACT CGG CGC T-3
DQB1*0503	5-TGC GGG GTG TGA CCA GAC-3 5-GCG GCG TCA CCG CCC GA-3
DQB1*0504	5-GTG CGG GGT GTG ACC AGA T-3 5-TGT TCC AGT ACT CGG CGC T-3

Specific IgE determination

Specific IgE antibodies to acid anhydride conjugated with human serum albumin were determined by RAST, as described earlier [7, 8]. Results were expressed as % specific binding (c.p.m. of test disc – c.p.m. of HSA reference disc) of total added radioactivity. A positive test was scored when the result exceeded the highest value of a non-exposed control group, i.e. > 1.5% binding [8].

Statistical analysis

Thirteen DR antigens and five DQ antigens were examined. Differences between the cases and their matched referents were analysed using conditional logistic regression, with results expressed as odds ratios (OR) and 95% confidence intervals (95% CI). Interactions between carriage of alleles

and exposure intensity were analysed by including an interaction term in each model. All analyses were done using SAS 6.12 (SAS Institute Inc., Cary, NC, USA) and Stata 6 (Stata Corporation, College Station, TX, USA) statistical software.

Results

The carriage of DR and DQ alleles by case status is summarized in Table 2. In the matched case-control study, there was a significantly increased carriage of DQ5 and DR1 alleles in the cases compared with the referents (OR = 4.3 and 3.0, respectively). The carriage frequencies of DR and DQ alleles in the total acid anhydride worker population (cases and referents, $n = 190$) were similar to a previously published control Scandinavian population ($n = 250$) Table 2 [12].

In the matched case-control study, DQ5 was more strongly associated with sensitization to HHPA, MHPA and MTHPA than was DR1, thus suggesting that the association was primarily with DQ5. For this reason, cases and referents who were DQ5, were further sub-typed. The DQ5 positive cases and referents were found to be either DQB1*0501, 0502 or 0503. There was a significantly increased carriage of DQB1*0501 in the cases compared with their referents (OR 3.0) (Table 3), whereas DQB1*0502 and 0503 were not significantly increased in the cases compared with the referents.

Workers from different sites are exposed to different acid anhydrides, namely HHPA and MHPA at site 1 and MTHPA at site 2. We, therefore, investigated whether DQ5 was associated with sensitization to the different acid anhydrides used at both sites. The prevalence of both DQ5 and DR1 with the development of IgE antibodies to

Table 2. Carriage of allele frequency in acid anhydride cases and referents

HLA	Total population with DNA samples <i>n</i> = 190		Matched case referent population <i>n</i> = 125				
	Cases <i>n</i> = 74 (%)	Referents <i>n</i> = 116 (%)	Matched groups: cases <i>n</i> = 52, referents <i>n</i> = 73		OR (95% CI)	Cases+referents <i>n</i> = 190 (%)	Control group* <i>n</i> = 250 (%)
			Case+ referents –	Case – referent+			
DR1	22 (30)	20 (17)	22	7	3.0 (1.2, 7.3)	22	18
DR15	15 (20)	31 (27)	9	17	0.4 (0.1, 1.1)	24	30
DR16	1 (1)	3 (3)	1	1	1.4 (0.1, 23.6)	2	1
DR3	16 (21)	30 (26)	14	19	0.7 (0.3, 1.6)	29	17
DR4	25 (34)	49 (42)	10	23	0.5 (0.2, 1.2)	39	37
DR11	3 (4)	13 (11)	4	8	0.5 (0.1, 1.9)	8	14
DR12	2 (3)	8 (7)	1	5	0.3 (0.03, 2.4)	5	6
DR13	16 (22)	36 (31)	15	17	0.8 (0.4, 1.6)	27	31
DR14	5 (7)	0	7	0	Infinite	3	6
DR7	7 (9)	16 (14)	3	8	0.4 (0.1, 1.5)	12	12
DR8	5 (7)	9 (8)	5	5	1.2 (0.3, 4.4)	7	10
DR9	7 (9)	6 (5)	7	2	3.5 (0.7, 18.1)	7	4
DR10	3 (4)	0	4	0	Infinite	2	2
DQ2	22 (30)	43 (37)	11	21	0.5 (0.2, 1.2)	34	23
DQ3	39 (53)	74 (64)	12	21	0.6 (0.3, 1.4)	59	67
DQ4	6 (8)	10 (9)	5	6	1.0 (0.3, 3.6)	8	10
DQ5	40 (54)	23 (20)	33	6	4.3 (1.7, 10.8)	33	27
DQ6	31 (42)	61 (52)	13	20	0.6 (0.3, 1.3)	48	48

*Control Swedish population [14].

Table 3. Carriage of DQB1*05 allele frequency in matched acid anhydride cases and referents

HLA-DQB1	Cases <i>n</i> (%)	Referents <i>n</i> (%)	Matched groups		OR (95% CI)
			Case+referents –	Case – referents +	
0501*	17 (33)	10 (14)	17	7	3.0 (1.2, 7.4)
0502*	2 (4)	1 (1)	2	0	Infinite
0503*	2 (4)	1 (1)	2	1	2.6 (0.2, 29.1)
0504*	0	0	0	0	0

Percentage are given on whole matched population (cases *n* = 52, referents *n* = 74)

*One sample was not available for sub-typing.

Table 4. HLA-DR1 and -DQ5 in cases and referents by category of site

HLA	Site	Cases <i>n</i> (%)	Referents <i>n</i> (%)	Matched groups		OR (95% CI)
				Case+control –	Case – control +	
DR1	1	12 (32)	7 (14)	12	5	3.2 (1.1, 9.3)
DR1	2	4 (27)	3 (13)	4	2	2.3 (0.4, 13.3)
DQ5	1	17 (46)	8 (16)	16	4	5.0 (1.7, 15.3)
DQ5	2	5 (33)	3 (13)	5	2	2.9 (0.5, 15.3)
DQ 0501	1	12 (33)	7 (14)	12	5	3.1 (1.1, 8.9)
DQ 0501	2	5 (33)	3 (13)	5	2	2.9 (0.5, 15.3)

Site 1 – workers exposed to hexahydrophthalic and methylhexahydrophthalic anhydrides.

Site 2 – workers exposed to methyltetrahydrophthalic anhydride.

Percentage are given on whole matched population (cases *n* = 52, referents *n* = 74).

anhydrides, was significantly raised amongst workers exposed to HHPA and MHHPA at site 1 (OR = 5.0 and 3.2, respectively) whereas there was only a trend present among workers exposed to MTHPA at site 2 which was not statistically significant (OR = 2.9 and 2.3, respectively) (Table 4). Further sub-typing of DQ5 demonstrated DQB1*0501 was significantly associated with development of anhydride-specific IgE antibodies in workers at site 1 (OR 3.1) but not in those workers at site 2 (OR = 2.9).

We found no evidence of an interaction between the intensity of acid anhydride exposure and strength of association between development of specific IgE antibody to the acid anhydride and HLA molecules DR1, DQ5 and DQB1*0501 (*P* = 0.94, 0.83, and 1.00, respectively).

Discussion

To date this is the largest study of HLA association with sensitization to acid anhydrides. In the matched case-control study, we established a significant association between DQ5 and DR1 and IgE sensitization to HHPA, MHHPA and MTHPA. DQ5 and DR1 are known to be in strong linkage disequilibrium. DQ5 was more strongly associated with sensitization to HHPA, MHHPA and MTHPA than was DR1. We, therefore, suggest that the association between HLA and acid anhydride sensitization is with DQ5, and in particular alleles of DQB1*0501, rather than with DR1 antigen.

A more detailed investigation of workers exposed to HHPA and MHHPA at site 1 and MTHPA at site 2 revealed that DQ5 was significantly associated with sensitization to both HHPA and MHHPA at site 1, OR = 5.0 (1.7, 15.3) and OR = 3.2 (1.1, 9.3), respectively, but there was only a trend

with MTHPA at site 2, OR = 2.9 (0.5, 15.3) and OR = 2.3 (0.4, 13.3). These associations attained statistical significance at site 1 but not at site 2, which may be due to several factors. Differences between the association of DQ5 at site 1 and 2 may merely reflect the larger number of workers exposed to HHPA and MHHPA at site 1 compared to site 2. However, it may imply that the chemical structure of MTHPA is sufficiently different from HHPA and MHHPA, such that it is presented on different HLA alleles.

A previous study demonstrated an association between DR3 and TMA and possibly TCPA, but not with PA [2]. The association of DR3 and sensitization to TMA and TCPA but not PA, indicates that small differences in the acid anhydride molecule result in presentation by different class II alleles. Previously, inhibition studies on the binding of specific IgE antibodies have indicated that HHPA, MHHPA and MTHPA but not TMA and TCPA are immunogenically similar [13]. It is, therefore, likely that differences in the chemical structures of HHPA, MHHPA and MTHPA would result in these molecules being presented on different HLA molecules to TMA and TCPA.

In this study, we were able to demonstrate that DQB1*0501 in particular, but also DQB1*0502 and DQB1*0503, were associated with sensitization to HHPA and MHHPA. There are several amino acids within DQB1*0501, 0502, 0503 which may be important in presenting the anhydrides. These positions include leucine at position 14, glycine at position 26, histidine at position 30, glycine at position 70, isoleucine at position 116 and serine at position 125. Comparison of the amino acid residues in the peptide binding pockets between the DQB1 antigens suggest that glycine at position 26 may be particularly important. Position 26 has been proposed to contribute to peptide binding pockets 6, 7 and 9 [14]. The more common alleles of DQB1 have either a small

hydrophobic or aromatic residue at position 26, whereas DQB1*05 has a small hydrophilic residue. This may act as a critical residue for the binding of HHPA and MHPA.

The associations between DR1 and DQ5 and sensitization to acid anhydrides were independent of exposure levels. This is in contrast to previous findings with complex platinum salts, where the influence of genetic susceptibility appeared greater at low-exposure levels [15]. The accuracy of the present exposure classification is supported by association with urinary metabolites, protein adducts [16, 17] and association of specific antibodies with symptoms [3, 4]. It is possible that average or cumulative exposure may have provided a more sensitive indicator of exposure; however, this data was not readily available for the cases and referents. The matching of cases and controls by duration of exposure may have confounded our ability to detect a genetic exposure interaction. Alternatively, the low-exposure category in this study might be too high to observe a gene-environment interaction, although the exposure levels in the low-exposure category were low relative to other factories within the industry.

Another factor which may have had implications for the lack of observed gene-environment interaction was the power of the study. Although the sample size was similar to previous study where gene-environment interaction was observed [15], the sample size may have been inadequate to determine gene-exposure interaction. Alternatively, there is no variation in strength of genetic susceptibility at different levels of exposure for these anhydrides.

We found no evidence of any protective alleles in the development of sensitization to acid anhydrides. HLA class I antigens A25 and A32 have been reported to be significantly different between sensitized and non-sensitized acid anhydride workers [18]; class I molecules were not typed in this study.

In conclusion, we have identified HLA-DQ5 as an antigen, which confers susceptibility to developing IgE antibodies to human serum albumin conjugates of HHPA, MHPA. These findings should be considered as hypothesis generating and be tested in a further separate population.

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