Genetic Variants in the Major Histocompatibility Complex Class I and Class II Genes Are Associated With Diisocyanate-Induced Asthma

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Objective: To investigate the association between single nucleotide polymorphisms (SNPs) located across the major histocompatibility complex and susceptibility to diisocyanate-induced asthma (DA). **Methods:** The study population consisted of 140 diisocyanate-exposed workers. Genotyping was performed using the Illumina GoldenGate major histocompatibility complex panels. **Results:** The *HLA-E* rs1573294 and *HLA-DPB1* rs928976 SNPs were associated with an increased risk of DA under dominant (odds ratio [OR], 6.27; 95% confidence interval [CI], 2.37 to 16.6; OR, 2.79, 95% CI, 0.99 to 7.81, respectively) and recessive genetic models (OR, 6.27, 95% CI, 1.63 to 24.13; OR, 10.10, 95% CI, 3.16 to 32.33, respectively). The *HLA-B* rs1811197, *HLA-DOA* rs3128935, and *HLA-DQA2* rs7773955 SNPs conferred an increased risk of DA in a dominant model (OR, 7.64, 95% CI, 2.25 to 26.00; OR, 19.69, 95% CI, 2.89 to 135.25; OR, 8.43, 95% CI, 3.03 to 23.48, respectively). **Conclusion:** These results suggest that genetic variations within HLA genes play a role in DA risk.

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Learning Objectives

- Discuss previous research on genetic factors potentially contributing to asthma caused by diisocyanates and other low-molecular-weight sensitizers.
- Summarize the new findings on single-nucleotide polymorphisms (SNPs) of the major histocompatibility complex (MHC) associated with diisocyanate-induced asthma (DA).
- Discuss the implications for the mechanism of DA and the role of genetic variations of human leukocyte antigen (HLA) genes.

Diisocyanates, low-molecular-weight reactive chemicals used in the production of paints and polyurethanes, are one of the most common causes of occupational asthma. Toluene diisocyanate (TDI), 4,4′-diphenylmethane diisocyanate (MDI), and hexamethylene diisocyanate (HDI) are the most commonly used isocyanates. Between 5% and 15% of workers with continuous long-term exposure to diisocyanates develop asthma.[1–3] Toluene diisocyanate alone was reported to account for between 2.9% and 13% of all occupational asthma cases in Korea.[4] Genetic association studies have underscored the importance of human leucocyte antigen (HLA) genes within major histocompatibility complex (MHC) as susceptibility loci for a number of complex diseases with an immune/inflammatory nature including occupational asthma.[5–7] Since both HLA class I and II molecules are involved in the presentation of antigens to T-cell receptors, genetic research has focused on identifying interindividual differences in their ability to bind peptides and influence T-cell recognition. Evidence has shown that certain HLA class II alleles contribute to the risk of asthma caused by diisocyanates and other low-molecular-weight sensitizers (eg, trimellic anhydride and platinum salts).[8–10] Earlier studies reported associations between the *HLA-DQB1* alleles and altered risk of diisocyanate-induced asthma (DA).[8,11] Recently, haplotypes including *HLA-DRB1*, *-DQB1*, and *-DPB1* alleles were found to be associated with an increased risk of TDI asthma in Koreans.[12,13] Hur et al [14] also reported an association between a haplotype carrying the *HLA-DRB1*, *-DQB1*, and *-DPB1* alleles and elevated serum-specific immunoglobulin G (IgG) levels in MDI-exposed workers.

Although the HLA complex is one of the most extensively studied regions in the human genome, other genes in the MHC region have not yet been sufficiently investigated with regard to disease association. The MHC, located on the short arm of chromosome 6 (6p21.3, 28 970 148- 33 883 424 bp), is one of the most polymorphic and gene-dense regions of the genome. This region spans nearly 4 Mb and encodes more than 180 highly polymorphic genes, many of which influence immune function, susceptibility to complex diseases, and the outcome of tissue transplantation.[15] In addition to genes in the HLA complex, several functionally important genes are

located in this region including the genes for complement proteins C4, C2, and Factor B, the cytokines tumor necrosis factor α and β, and *TAP* (antigen peptide transporter) genes that function in antigen processing.

The dense genetic organization and extensive linkage disequilibrium (LD) patterns of the region complicate the search for susceptibility alleles. Although various MHC variants have been shown to be involved in susceptibility to autoimmune, infectious, and inflammatory diseases, thus far, only a limited number of HLA genes have been examined with respect to DA. This is the first study investigating the association of single nucleotide polymorphisms (SNPs) located across the entire MHC region with DA in a well-characterized worker population using microarray technology.

MATERIALS AND METHODS

Subjects

The study population consisted of 140 workers exposed to diisocyanates (HDI, MDI, and TDI). Of these, 73 were diagnosed with DA (DA+) on the basis of a positive specific inhalation challenge (SIC) test and 67 were asymptomatic workers (AWs) exposed to HDI. Symptomatic subjects were recruited from occupational pulmonary disease clinics located in Canada (Hôpital du Sacré-Coeur de Montréal, Montréal, 124 subjects; Laval Hospital, Sainte-Foy,12 subjects; University Health Network, Toronto, Ontario, 2 subjects) and Spain (Fundación Jiménez Díaz, Madrid, 2 subjects). The subjects underwent SIC testing with the appropriate work-relevant diisocyanate chemicals according to previously described protocols.[16,17] Patients were classified as DA+ on the basis of their positive response to SIC. A decrease in FEV_1 (forced expiratory volume in the first second of expiration) of at least 20% from prechallenge baseline during the early and/or late asthmatic response was defined as a positive SIC test. The AW controls were recruited in Quebec, Canada, from HDI-exposed painters and evaluated by occupational history, spirometry, and skin prick testing. Data regarding age, sex, ethnicity, smoking status, and time of exposure were collected by questionnaire. Atopy was evaluated by skin prick testing to common aeroallergens, defined by a positive reaction of at least 3 mm greater than saline control for at least one allergen. Antibodies were detected by isotype-specific enzyme-linked immunosorbent assay tests, as previously described.[18] Whole blood was collected for genetic testing. All subjects provided written informed consent, and the study protocol was approved by institutional review boards of National Institute for Occupational Safety and Health and each participating institution.

Genotyping

Genomic DNA was extracted from whole blood samples by using the QIAamp blood kit (QIAGEN Inc, Chatsworth, CA). Genotyping was performed according to the standard protocol provided by Illumina using the MHC Panel Set and Golden Gate protocol (Illumina Inc, San Diego, CA). The MHC SNP set consisted of two oligonucleotide pools, MHC Mapping Panel and MHC Exon-Centric Panel for 1228 and 1293 SNP loci, respectively. Both panels cover 2360 independent loci spaced at an average of 2.08 kb (range, 0.005 to 71.05 kb). Genotyping was performed in a 16-well format universal BeadChips. A total of 250 ng to 1 μg DNA was used for each assay, depending on the source. Genotypes were auto called using the BeadStudio software.

The genotype confidence score of the assay was set to 0.5. Data quality was assessed by controlling for discrepancies between 161 overlapping SNPs in the two panels. SNPs with more than two discrepant calls were removed from further analysis. In the remaining overlapping SNPs, consistency was 99.9%. Replicate sample comparisons within and across DNA genotyping plates also demonstrated high agreement (data not shown). Alleles that were not called in a sample were coded as missing in the analysis. A total of 140 samples and 2202 SNPs passed our quality control criteria and included in the final analysis.

Statistical Analyses

All statistical analyses were conducted using either SAS/STAT for Windows or R programming within Bioconductor. Comparisons between groups on demographic variables were analyzed using two-sample *t* tests and chi-squared tests for continuous and categorical variables, respectively. Initial analyses, including tests for Hardy-Weinberg Equilibrium, allele, and genotype frequencies, were calculated using the Fisher exact test. A Bonferroni adjustment for multiple comparisons from the array was performed on the 2 by 3 genotype and 2 by 2 allele frequency tables. Unadjusted odds ratios (OR) were calculated using contingency tables. Adjusted ORs were calculated using logistic regression while adjusting for age, exposure time (months), smoking (current/ex/never), pack years, height, sex, and atopic status. Since underlying genetic models are unknown a priori, the association between each SNP and DA status was analyzed using three genetic models. These include a dominant model (comparing homozygous wild-type genotype with variant allele-carrying genotypes), recessive model (comparing wild-type allele-carrying genotypes with homozygous variant genotype), and an additive model. Prior to the final analyses, we utilized the MICE algorithm (Multiple Imputations by Chained Equations) to impute missing data. Three independent imputations were generated and age, sex, height, exposure duration, smoking, and atopy were appended to each data set. There were no substantive differences between the 3 imputations and we present the results from the first imputation. SNAP2 tools were used to update annotations of significant SNPs according to dbSNP135 and to find proxy SNPs within 500 kb based on LD and physical distance.[19] RegulomeDB was used to annotate SNPs with known and predicted regulatory elements.[20]

RESULTS

Subject Characteristics

The demographic characteristics of the study groups included in the statistical analyses are described in Table 1. A total of 92% of the symptomatic workers diagnosed with DA (DA+) and 99% of the asymptomatic exposed workers (AW) were white French Canadians. Mean age was higher in the DA+ group than in AW controls (42.4 vs 30.0 years). While the DA+ group consisted of subjects exposed to

TABLE 1. The Demographic Characteristics of the Study Groups

	AWs ($n = 67$)	DA+ Cases ($n = 73$)
Age (mean ± SE)	30.0 ± 0.93	42.4 ± 1.32*
Sex (female/male)	5/62	10/63
Exposure (mean months ± SE)	63.4 ± 2.71	146.3 ± 16.36*
Exposure (HDI; MDI; TDI)	67 HDI	39; 15; 18
Ethnicity (%, French Canadian)	99	92
Atopy (positive/negative)	36/27	41/32
Smoking Status (cur/ex/never)	26/13/28	11/26/36*
Smoking (pack/years ± SE)	6.2 ± 1.11	9.4 ± 1.66

*$P < 0.05$.

AW, asymptomatic workers; DA+, symptomatic workers diagnosed with DA; HDI, hexamethylene diisocyanate; MDI, 4,4′-diphenylmethane diisocyanate; TDI, toluene diisocyanate.

TABLE 2. Distribution of Genotype Frequencies Between the Groups

Gene	SNP	Position	Location	Genotype	DA+ ($n = 73$) N (%)	AWs ($n = 67$) N (%)	Fisher Exact Test P (DA+ vs AWs)
HLA-B	rs1811197	-2725	flanking_5UTR	GG	40 (54.8)	62 (92.5)	<0.0001
				GA	29 (39.7)	5 (7.5)	
				GG	4 (5.5)	0 (0)	
HLA-E	rs1573294	-70894	>10 kb coding	AA	24 (32.9)	49 (73.1)	<0.0001
				AG	21 (28.8)	14 (20.9)	
				GG	28 (38.3)	4 (6.0)	
HLA-DOA	rs3128935	-949	flanking_3UTR	TT	47(64.4)	65 (97.0)	<0.0001
				CT	19 (26.0)	2 (3.0)	
				CC	7(9.6)	0 (0)	
HLA-DQA2	rs7773955	-2444	flanking_5UTR	TT	26 (35.6)	56 (83.6)	<0.0001
				TC	32 (43.8)	11 (16.4)	
				CC	15 (20.6)	0 (0)	
HLA-DPB1	rs928976	-499	intron	CC	44 (60.3)	31 (46.3)	<0.0001
				CT	15 (20.5)	29 (43.3)	
				TT	14 (19.2)	7 (10.4)	

AW, asymptomatic workers; DA+, symptomatic workers diagnosed with DA; HLA, human leukocyte antigen; SNP, single nucleotide polymorphism.

HDI, MDI, and TDI (39, 15, and 18, respectively), the AW controls were exposed to HDI in the workplace. The AW controls had less duration of exposure to isocyanates than the DA+ group (63.4 vs 146.3 months). The frequency of atopy was similar in groups (54% in DA+ and 56% in AWs). The number of pack years was higher in the DA+ group than in AW controls (9.4 vs 6.2 years). The allele frequencies in the control population were in Hardy–Weinberg equilibrium (data not shown).

Genotype Distribution and Genetic Models

SNPs in *HLA-E, HLA-B, HLA-DOA, HLA-DQA2*, and *HLA-DPB1* genes (rs1573294, rs1811197, rs3128935, rs7773955, and rs928976, respectively) remained significantly associated with DA after the Bonferroni adjustment for multiple testing. Table 2 shows the distribution of genotypes in the study population. There were no SNPs that were statistically significant under an additive model and thus only the dominant and recessive models are presented (Table 3). The rs1573294 SNP was associated with an increased risk of DA under dominant and recessive genetic models (OR, 6.27, 95% confidence interval [CI], 2.37 to 16.61; OR, 6.27, 95% CI, 1.63 to 24.13, respectively). The *HLA-B* rs1811197, *HLA-DOA* rs3128935, and *HLA-DQA2* rs7773955 SNPs conferred an increased risk of DA in a dominant model (OR, 7.64, 95% CI, 2.25 to 26.00; OR, 19.69, 95% CI, 2.89 to 135.25; OR, 8.43, 95% CI, 3.03 to 23.48, respectively). The *HLA-DPB1* rs928976 SNP was also associated with higher risk of DA under dominant and recessive genetic models (OR, 2.79, 95% CI, 0.99 to 7.81; and OR, 10.10, 95% CI, 3.16 to 32.33). No association was found between non-HLA gene variants and DA.

Regulatory Information for Significant Associations

Five unique significant SNPs were used as inputs to the SNP Annotation and Proxy Search tool to find highly correlated SNPs within 500 kb (using an r^2 of 1).[20] This led to the identification of an additional 34 correlated SNPs using data from the International HapMap Project.[21] The total set of 39 SNPs was then used as inputs to the RegulomeDB[19] web resource, which integrates data from the ENCODE projects and other data sources regarding various types of functional assays including DNaseI-seq, ChIP-seq, RNAseq, and eQTL analyses. RegulomeDB showed that SNP rs1811197 has the potential to affect antigen binding and is linked to expression of a gene target. We were unable to find information pertaining to the possible functional role for the other significant SNPs.

DISCUSSION

In this study, significant associations were found between SNPs mapped to the MHC class I (*HLA-E, HLA-B*) and class II (*HLA-DOA, HLA-DQA2*, and *HLA-DBP1*) genes and DA in a group of exposed workers. Serum specific antibodies for diisocyanate antigens and the presence of eosinophils and activated T-cells in bronchial biopsies of workers with DA suggest a mechanistic role for antigen-specific immunological mechanisms.[22–25] T cells are activated by the interaction of the T-cell receptor with antigenic peptides complexed to MHC molecules. The class I (HLA-A, -B, -C, -E, -F, and -G) and class II (HLA-DR, -DQ, -DM, and -DP) MHC molecules are responsible for the presentation of antigenic peptides to $CD8^+$ and $CD4^+$ T cells, respectively.[26] Since HLA molecules are highly polymorphic, specific peptide epitopes presented to T cells widely vary across the HLA genes and their alleles. The average SNP density varies from 1 to >60 SNPs per kb across the MHC region and these variations are located mainly in the class I and class II HLA molecules. Therefore, it is plausible that genetic variations in the HLA genes markedly influence individual susceptibility.

In our analysis, two SNPs in MHC class I genes, *HLA-B* and *HLA-E*, were independently associated with DA. The carriage of the minor allele for *HLA-B* rs1811197 SNP was associated with an increased risk of DA. Functional annotation of SNPs using RegulomeDB showed that the rs1811197 affects the expression level of the *HLA-C* gene. *HLA-B* and *HLA-C* are classical MHC class I molecules that play a central role in antigen processing/presentation and immune regulation. Data on the association between *HLA-B* and *HLA-C* SNPs/alleles and asthma are restricted to a few studies. Beghe et al[27] examined possible associations of class I (*HLA-A*, *HLA-B*, and *HLA-C*) alleles with DA in 142 subjects with TDI-asthma and a comparator group of 50 asymptomatic exposed subjects. No significant associations were identified between HLA class I alleles and TDI-asthma.[27] Nevertheless, associations between different *HLA-B*

TABLE 3. Association of HLA SNPs With DA Under Different Genetic Models

		Dominant Model			Recessive Model		
Gene	dbSNP ID	Unadjusted OR (95% CI)	P	Adjusted* OR (95% CI)	P	Unadjusted OR (95% CI)	P	Adjusted* OR (95% CI)	P
HLA-E	rs1573294	5.55 (2.68, 11.51)	<0.0001	6.27 (2.37, 16.61)	0.0002	9.80 (3.21, 29.89)	<0.0001	6.27 (1.63, 24.13)	0.0076
HLA-B	rs1811197	10.23 (3.68, 28.40)	<0.0001	7.64 (2.25, 26.00)	0.0001	8.74† (0.46, 165.5)	0.0519	‡	
HLA-DOA	rs3128935	17.98 (4.07, 79.48)	<0.0001	19.69 (2.89,135.25)	0.0024	15.22† (0.85, 271.9)	0.0093	‡	
HLA-DQA2	rs7773955	9.20 (4.12, 20.57)	<0.0001	8.43 (3.03, 23.48)	<.0001	35.76† (2.09, 610.9)	<0.0001	‡	
HLA-DPB1	rs928976	3.63 (1.71, 7.72)	0.0009	2.79 (0.99, 7.81)	0.0511	13.00 (5.22, 32.39)	<0.0001	10.10 (3.16, 32.33)	<0.0001

*Logistic regression models were adjusted for age, sex, atopy, height, exposure duration, and smoking.
†Logit estimators used to adjust for cells with 0 count.
‡Models failed to converge due to cells with 0 counts.
AW, asymptomatic workers; CI, confidence interval; DA+, symptomatic workers diagnosed with diisocyanate asthma; HLA, human leucocyte antigen; OR, odds ratio; SNP, single nucleotide polymorphism.

alleles (*HLA-B8, B12, B16 and Bw61*) and asthma phenotypes have been reported.[28–30] *HLA-E*, a nonclassical MHC class I molecule, plays an important role in both natural and acquired immune responses by binding peptides derived from the leader sequence of other HLA class I molecules. We found rs1573294 SNP that mapped to *HLA-E* to be associated with an increased risk of DA. Although the functional consequence of this SNP is unknown, this association suggested a possible involvement of the *HLA-E* region in susceptibility to DA. A functional role for MHC class I molecules in the elicitation of DA has not been defined. Hypothetically, it is possible that reactive diisocyanates, known to penetrate cell membranes, could significantly modify endogenous cytosolic proteins, which could then be taken up by proteasomes and digested into antigenic peptides. Peptides could be transported to the endoplasmic reticulum, bound and captured by class I molecules, and transported to the cell surface for antigen presentation.

The HLA class II region is one of the most gene-dense regions in the human genome and is associated with many diseases and the dense LD pattern can complicate the identification of functional variants. We found a significant association between SNPs mapping to *HLA-DPB1, HLA-DQA2*, and *HLA-DOA* genes and increased risk of DA. The *HLA-DPB1* rs928976 SNP has not been previously associated with any disease risk, and no functional data have been reported to date. Nevertheless, previous studies found a significant contribution of other *HLA-DPB1* alleles to asthma risk. A haplotype including *DPB1*05 (DRBI*15-DPB1*05)* was strongly associated with TDI-induced asthma in a Korean population[13] and the allelic frequencies of *HLA DQB1*06-DPB1*05* and *DRB1*15-DQB1*06-DPB1*05* were significantly higher in TDI asthmatic patients. The same investigators later conducted another study that included subjects with TDI asthma, asymptomatic exposed controls, and unexposed normal controls using high-resolution HLA analysis. The frequency of the *HLA DRB1*1501-DQB1*0602-DPB1*0501* haplotype was found to be significantly higher in TDI asthmatic patients than in asymptomatic exposed and normal controls.[12] Genetic variants in the HLA-DP locus were also associated with the risk of other asthma phenotypes, including allergic, pediatric, and aspirin-intolerant asthma.[31–33] These reports support our finding and suggest involvement of *HLA-DBP1* in asthma pathogenesis.

We also identified two SNPs mapping to the *HLA-DOA* and *DQA2* genes that were associated with DA (rs3128935 and rs7773955, respectively). HLA-DO is a nonclassical class II heterodimer consisting of α and β chains, which are encoded by the *HLA-DOA* and *HLA-DOB* genes. *HLA-DOA* has been proposed to have functional implications in autoimmunity and can inhibit the activity of *HLA-DM* genes in vitro that regulates the antigen loading and presentation of specific peptides.[34] A recent study identified an SNP in *HLA-DOA* gene (rs9276977) significantly associated with rheumatoid arthritis in African Americans.[35] Nevertheless, there are no studies showing association between *HLA-DOA* SNPs and asthma phenotypes.

HLA-DQ consists of α and β chains that are encoded by *HLA-DQA1* and *HLA-DQB1*, respectively. These two loci are adjacent to each other and in close genetic linkage to *HLA-DR*. *HLA-DQ* was the first asthma-susceptibility locus to be identified and plays a major role in peptide loading of MHC II molecules.[36] Our results showed a significant association between *HLA-DQA2* rs7773955 SNP and DA risk. Although the functional role of this SNP is not known, previously reported associations between HLA-DQ alleles and asthma phenotypes suggest a role for DQ in the asthmatic process. Bignon et al[11] found that the *HLA DQB1*0503* and the allelic combination *DQB1*0201/0301* were associated with susceptibility to DA. On the contrary, the *DQB1*0501* allele and the *DQA1*0101-DQB1*0501-DR1* haplotype were reported to be protective.[11] Although not replicated in other European populations, Mapp et al [8,37] confirmed these results and also reported an association between *HLA-DQB1*0503*

allele with an aspartic acid at residue 57 and TDI asthma.[38] Previous genome wide association studies have also shown associations between SNPs in the HLA-DQ/DR region and asthma phenotypes.[39,40]

The major strengths of this study include a well-defined phenotype and selection of candidate regions based on their functional role in disease pathogenesis. In addition, genetic associations were tested while adjusting for potential confounding factors and results were corrected for multiple comparisons. The major limitations include small sample size due to the relative rarity of DA compared with other types of asthma; however, rigorous phenotypic characterization in this population helps maximize the discriminatory potential between study groups. Another limitation is that the controls were younger and had shorter exposure period than cases. This was unintentional due to difficulty in the recruitment of age-matched workplace controls. Nevertheless, it has been reported that nearly 40% and 60% of subjects exposed to isocyanates become symptomatic within 1 year and after 5 years of exposure, respectively.[41]

Taken together, this study showed novel associations between SNPs in MHC class I (*HLA-E, HLA-B*) and class II (*HLA-DOA, HLA-DQA2*, and *HLA-DBP1*) genes and DA. The present results are consistent with the hypothesis that an immunological mechanism is involved in DA and that genetic variations within HLA genes play a major role in DA risk. Identification of significant polymorphisms and their allelic variations within the MHC is potentially important as the structural diversity of the MHC alleles influences peptide binding and controls disease susceptibility. For example, hypersensitivity syndrome induced by antiviral drug abacavir is strongly associated with the *HLA-B*5701* allele that excluding those with this particular allele prior treatment is effective in preventing drug reactions.[42] Further studies are needed to validate the results reported herein and identify causative alleles behind these associations using high-resolution mapping.

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