

Letter to the Editor

Hexamethylene diisocyanate asthma is associated with genetic polymorphisms of CD14, IL-13, and IL-4 receptor α

To the Editor:

Diisocyanates are among the most common causes of occupational asthma. However, susceptibility factors and immune biomarkers of diisocyanate asthma (DA) have not been clearly defined. For example, serum diisocyanate antigen-specific IgE and IgG have been extensively investigated, but these immunoassays lack diagnostic accuracy in identifying workers with confirmed DA.¹⁻³ Various genetic variants have been identified as risk factors for DA in association studies. Certain HLA class II alleles and single nucleotide polymorphisms (SNPs) of antioxidant enzymes (eg, glutathione-S-transferases, N-acetyl transferases) have been associated with confirmed DA, although these findings have not yet been replicated in multiple populations.⁴

In 2006, we first reported that DA confirmed by specific inhalation challenge (SIC) testing was significantly associated with cytokine genotype combinations of IL-4 receptor α (*IL4RA*), *IL13*, and *CD14* SNPs, but exclusively in hexamethylene diisocyanate (HDI)-exposed workers.⁵ In this report, we confirm the aforementioned genotype associations in an expanded group of workers with confirmed DA compared with diisocyanate-exposed workers without DA.

A total of 368 diisocyanate-exposed workers were recruited by clinical investigators at 4 occupational disease clinics (Hôpital du Sacré-Cœur de Montréal, Montreal, Quebec, Canada; Hôpital Laval, Sainte-Foy, Quebec, Canada; University Health Network, Toronto, Ontario, Canada; and Fundacion Jimenez Diaz, Madrid, Spain) and included 103 diagnosed with DA (DA⁺) on the basis of a positive SIC test, 115 symptomatic workers with negative SIC tests (DA⁻), and 150 HDI-exposed asymptomatic control spray paint workers (ie, healthy subjects not exhibiting respiratory symptoms). Blood samples were obtained for DNA extraction and genotyped for *IL4RA* (I50V), *IL4RA* (Q551R), *IL4RA* (E375A), *IL13* (R110Q), and *CD14* (C159T) SNPs, as previously described.⁵ Subjects were predominantly male (91%), white (99%), and of European descent (90.8% French Canadian, 2.7% English Canadian, 1.9% Spanish). Of 103 workers with DA, 50, 22, and 31 were exposed to HDI, methylene diphenyldiisocyanate (MDI), and toluene diisocyanate (TDI), respectively. Of the 115 DA⁻ symptomatic workers, 91, 18, and 6 were exposed to HDI, MDI, and TDI, respectively, whereas all asymptomatic controls were exposed to HDI. The duration of workplace exposure in months (mean $\pm$ SD) was 137 ± 13.9 for DA⁺, 157.9 ± 14.2 for DA⁻, and 65.6 ± 2.2 for asymptomatic HDI workers.

All SNPs studied were in Hardy-Weinberg equilibrium (χ^2 tests, all $P > .10$). No significant associations were identified (by the χ^2 test) between DA and individual alleles or genotypes of the candidate SNPs for *IL4RA*, *IL13*, or *CD14*. Genotypes were dichotomized for further statistical analyses as follows: *IL4RA* (I50V), II versus IV or VV; *IL4RA* (Q551R), QQ versus QR or RR; *IL4RA* (E375A), EE versus EA or AA; *IL13* (R110Q), RR versus RQ or QQ; and *CD14* (C159T), CT versus CC or TT. Combinations of genotypes were also dichotomized to compare the indicated combination with all other possible combinations. For

example, in Table I, the combination of *IL4RA* and *IL13* RR compared *IL4RA* (I50V) = II and *IL13* (R110Q) = RR to all other combinations of *IL4RA* (I50V) and *IL13* (R110Q). Logistic regression analysis revealed significant interactions of diisocyanate exposure (HDI vs MDI, TDI) with specific genotype combinations (ie, *IL4RA* II + *IL13* RR; *IL4RA* II + *CD14* CT; and *IL4RA* II + *IL13* RR + *CD14* CT) for distinguishing confirmed DA status (DA⁺) compared with SIC-negative workers (DA⁻), after adjusting for significantly associated demographic variables (Table I).

In logistic regression analyses comparing HDI-exposed DA⁺ workers ($n = 50$) with HDI-exposed DA⁻ workers ($n = 91$), DA remained significantly associated with *IL4RA* II + *CD14* CT and *IL4RA* II + *IL13* RR + *CD14* CT genotype combinations (Table I) after adjustment for smoking status and sex. When comparing HDI-exposed DA⁺ workers ($n = 50$) with asymptomatic HDI-exposed workers ($n = 150$), the association between DA and the *IL4RA* II + *CD14* CT and *IL4RA* II + *IL13* RR + *CD14* CT genotype combinations approached statistical significance ($P < .10$) after adjustment for age at diagnosis and smoking status (Table II).

These results in an expanded group of workers confirm our original findings of significant associations of DA with *IL4RA* (I50V), *IL13* (R110Q), and *CD14* (C159T) genotype combinations modified by exposure to HDI.⁵ Unique to this report is the finding that 2 genotype combinations remain significantly associated with DA compared with DA⁻ workers, and a similar, but not statistically significant, association was observed when compared with an asymptomatic cohort of HDI-exposed workers. Significant associations between genotype and occupational asthma were found only after adjustment for work-relevant diisocyanate exposure (ie, HDI vs TDI, MDI). The reason for restriction of this finding to the HDI-exposed population is unknown and may be an artifact of the greater numbers of HDI-exposed subjects available for statistical analysis.

This study used a case-control design with a candidate gene approach. The rarity of diisocyanate asthma and the relatively small numbers of subjects able to be recruited compared with genetic studies of nonoccupational asthma are limitations of this study and hindrances to future replication studies. However, the issue of small group sizes could be counterbalanced by the ability to define the DA phenotype precisely by using objective SIC tests with work-relevant diisocyanate chemicals. The role of these genotype combinations in the pathogenesis of DA is unknown and awaits functional characterization. Nevertheless, susceptibility genes associated with T_H2-cell differentiation (eg, *IL4RA*, *IL13*) and innate immunity (*CD14*) have been extensively investigated in nonoccupational asthma.⁶ The *IL13* (R110Q) SNP has been associated with asthma and airway hyperresponsiveness, and *IL4RA* variant V and R alleles (I50V and Q551R, respectively) have been associated with asthma and atopy.^{7,8} No such associations were detected in the current study of workers not enriched with atopic subjects.

In summary, we have confirmed a previous observation in expanded groups of workers: a reported association between genotype combinations associated with T_H2 and innate immunity and diisocyanate-induced asthma caused by HDI. Replication of these results in other background populations will be necessary to define the possible value of these genetic markers for risk assessment.

TABLE I. Logistic regression analyses of associations of genotype or genotype combinations and exposure (HDI vs MDI and/or TDI) with occupational asthma confirmed by SIC-positive (DA⁺) versus SIC-negative (DA⁻) workers in all diisocyanate-exposed workers (**A**), and of genotype or genotype combinations with DA⁺ versus DA⁻ in HDI-exposed workers (**B**), after adjusting for significant demographic characteristics

Genotype or genotype combinations of <i>IL4RA</i> (I50V), <i>IL13</i> (R110Q), and <i>CD14</i> (C159T) SNPs	<i>P</i> values			OR (95% CI) for genotype among diisocyanate-exposed workers
	Genotype or genotype combination main effect	Exposure main effect, HDI vs MDI, TDI	Genotype or genotype combination by exposure interaction	
A. All workers				
<i>IL4RA</i> II*	.810	.0001	.100	1.57 (0.74-3.32)†
<i>IL4RA</i> II and <i>IL13</i> RR	.578	.005	.051	1.66 (0.66-4.17)†
<i>IL4RA</i> II and <i>CD14</i> CT	.895	.033	.006	2.98 (1.25-7.09)†
<i>IL4RA</i> II and <i>IL13</i> RR and <i>CD14</i> CT	.801	.564	.003	3.55 (1.19-10.55)‡
B. HDI workers only				
<i>IL4RA</i> II	.292			1.51 (0.70-3.28)†
<i>IL4RA</i> II and <i>IL13</i> RR	.340			1.58 (0.62-4.06)†
<i>IL4RA</i> II and <i>CD14</i> CT	.015			3.08 (1.25-7.60)†
<i>IL4RA</i> and <i>IL13</i> RR and <i>CD14</i> CT	.019			3.86 (1.26-11.98)§

OR, Odds ratio.

*Each row represents a separate logistic model. Genotypes are dichotomized so that *IL4RA* II compares the II genotype to the IV or VV genotypes, *IL13* compares the RR genotype to the RQ or QQ genotypes, and *CD14* CT compares the CT genotype to the CC or TT genotype. Combinations of genotypes compare the indicated SNP combination with all other combinations. For example, *IL4RA* II and *IL13* RR compare the combination of *IL4RA* (I50V) = II and *IL13* (R110Q) = RR with all other combinations of *IL4RA* (I50V) and *IL13* (R110Q).

†Adjusted for smoking.

‡Adjusted for smoking and ethnicity.

§Adjusted for smoking and sex.

TABLE II. Logistic regression analyses in HDI-exposed workers of associations of genotype or genotype combinations with SIC-positive workers (DA⁺) versus asymptomatic workers (controls) after adjusting for significant demographic variables

Genotype or genotype combination of <i>IL4RA</i> (I50V), <i>IL13</i> (R110Q), and <i>CD14</i> (C159T) SNPs	P value	OR (95% CI) for genotype†
HDI-exposed workers only		
<i>IL4RA</i> *	.151	1.68 (0.83-3.40)
<i>IL4RA</i> II and <i>IL13</i> RR	.345	1.51 (0.64-3.54)
<i>IL4RA</i> II and <i>CD14</i> CT	.087	2.18 (0.89-5.30)
<i>IL4RA</i> II and <i>IL13</i> RR and <i>CD14</i> CT	.093	2.65 (0.85-8.23)

OR, Odds ratio.

*Each row represents a separate logistic model. Genotypes are dichotomized and analyzed as explained in Table I.

†Adjusted for age at diagnosis and smoking.

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