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Hexamethylene Diisocyanate Asthma is Associated with Genetic Polymorphisms of CD14, IL-13, and IL-4RA

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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, 2, 3]. Various genetic variants have been identified as risk factors for DA in association studies. Certain HLA class II alleles and SNPs of antioxidant enzymes (e.g., 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 interleukin 4 receptor alpha (IL4RA), interleukin 13 (IL13) and CD14 single nucleotide polymorphisms (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 when compared to diisocyanate exposed workers without DA.

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A total of 368 diisocyanate-exposed workers were recruited by clinical investigators at four occupational disease clinics (Hôpital du Sacré-Cœur de Montréal, Montreal, Canada; Hôpital Laval, Sainte-Foy, Canada; University Health Network, Toronto, Canada; and Fundacion Jimenez Diaz, Madrid, Spain) and included: 103 diagnosed with DA (DA+) based on a positive SIC test; 115 symptomatic workers with negative SIC tests (DA−); and 150 HDI-exposed asymptomatic control spray paint workers (i.e, 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%), Caucasian (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, MDI, and TDI, respectively, Of the 115 DA− symptomatic workers, 91, 18, and 6 were exposed to HDI, MDI, and TDI, respectively, while all asymptomatic controls were exposed to HDI. The duration of workplace exposure in months (mean ± 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 (chi-square tests, all $p>0.10$). No significant associations were identified (by the chi-square 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 vs. IV or VV; IL4RA (Q551R), QQ vs. QR or RR; IL4RA (E375A), EE vs. EA or AA; IL-13(R110Q), RR vs. RQ or QQ; CD14 (C159T), CT vs. CC or TT. Combinations of genotypes were also dichotomized to compare the indicated combination to all other possible combinations. For example, in Table I, the combination of IL4RA II and IL13 RR compared IL4RA (I50V) = II and IL13 (R110Q) = RR to all other combinations of IL4RA (I50V) and IL-13(R110Q). Logistic regression analysis revealed significant interactions of diisocyanate exposure (HDI vs. MDI, TDI) with specific genotype combinations (i.e., IL4RA II + IL13RR; IL4RA II + CD14 CT; and IL4RA II + IL13 RR + CD14 CT) for distinguishing confirmed DA status (DA+) in comparison to 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 gender. 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 ($p < 0.10$) 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 two genotype combinations remain significantly associated with DA when 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 (i.e., 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 employed a case control design using 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 non-occupational asthma is a limitation of this study and a hindrance to future replication studies. However, the issue of small group sizes could be counter-balanced by the ability to precisely define the DA phenotype 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 Th2 helper cell differentiation (e.g., IL4RA, IL13) and innate immunity (CD14) have been extensively investigated in non-occupational 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 present study of workers which were 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 Th2 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.

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Abbreviations

DA	diisocyanate asthma, confirmed by a specific inhalation challenge test
DA-	symptomatic worker, in which diisocyanate asthma has been ruled out by a specific inhalation challenge test
HDI	hexamethylene diisocyanate
MDI	methylene diphenyldiisocyanate
TDI	toluene diisocyanate
SIC	specific inhalation challenge test
SNP	single nucleotide polymorphism

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Capsule Summary

Diisocyanate asthma is associated with IL4RA (I50V), IL13 (R110Q) and CD14 (C159T) genotype combinations in workers exposed to hexamethylene diisocyanate.

TABLE I

Logistic regression analyses of associations of genotype or genotype combinations and exposure (HDI vs. MDI and/or TDI) with OA confirmed by specific inhalation challenge (DA+) versus specific inhalation challenge negative (DA−) workers in all diisocyanate exposed workers (Part A), and of genotype or genotype combinations with OA in HDI exposed workers (Part B), after adjusting for significant demographic characteristics.

Genotype or genotype combinations of IL4RA (I50V), IL13 (R110Q), and CD14 (C159T) SNPs.	P-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				
IL4RA II	0.810	0.0001	0.100	1.57 (0.74, 3.32)[a]
IL4RA II and IL13 RR	0.578	0.005	0.051	1.66 (0.66, 4.17)[a]
IL4RA II and CD14 CT	0.895	0.033	0.006	2.98 (1.25, 7.09)[a]
IL4RA II and IL13RR and CD14 CT	0.801	0.564	0.003	3.55 (1.19, 10.55)[b]
B. HDI workers only				
IL4RA II	0.292			1.51 (0.70, 3.28)[a]
IL4RA II and IL13 RR	0.340			1.58 (0.62, 4.06)[a]
IL4RA II and CD14 CT	0.015			3.08 (1.25, 7.60)[a]
IL4RA II and IL13RR and CD14 CT	0.019			3.86 (1.26, 11.98)[c]

Note that genotypes are dichotomized so that IL4RA II compares the II genotype to the IV or VV genotypes; IL13 RR compares the RR genotype to the RQ or QQ genotypes; CD14 CT compares the CT genotype to the CC or TT genotype. Combinations of genotypes compare the indicated SNP combination to all other combinations. For example, IL4RA II and IL13 RR compares the combination of IL4RA (I50V) = II and IL-13 (R110Q) = RR to all other combinations of IL4RA (I50V) and IL-13 (R110Q).

[a] Adjusted for smoking

[b] Adjusted for smoking and ethnicity

[c] Adjusted for smoking and gender

TABLE II

Logistic regression analyses in HDI exposed workers of associations of genotype or genotype combinations with specific inhalation challenge positive workers (DA+) versus asymptomatic workers (Controls), after adjusting for significant demographic variables.

Genotype or genotype combination of IL4RA (I50V), IL13 (R110Q), and CD14 (C159T) SNPs.	P-value	OR (95%CI) for genotype[a]
HDI exposed workers only		
IL4RA II	0.151	1.68 (0.83, 3.40)
IL4RA II and IL13 RR	0.345	1.51 (0.64, 3.54)
IL4RA II and CD14 CT	0.087	2.18 (0.89, 5.30)
IL4RA II and IL13RR and CD14 CT	0.093	2.65 (0.85, 8.23)

Note that genotypes are dichotomized so that IL4RA II compares the II genotype to the IV or VV genotypes; IL13 RR compares the RR genotype to the RQ or QQ genotypes; CD14 CT compares the CT genotype to the CC or TT genotype. Combinations of genotypes compare the indicated SNP combination to all other combinations. For example, IL4RA II and IL13 RR compares the combination of IL4RA (I50V) = II and IL-13 (R110Q) = RR to all other combinations of IL4RA (I50V) and IL-13 (R110Q).

[a] Adjusted for age at diagnosis and smoking