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CTNNA3 (α-Catenin) Gene Variants Are Associated With Diisocyanate Asthma: A Replication Study in a Caucasian Worker Population

David I. Bernstein,*[,1] Michael Kashon,† Zana L. Lummus,* Victor J. Johnson,‡ Kara Fluharty,† Denyse Gautrin,§ Jean-Luc Malo,§ André Cartier,§ Louis-Philippe Boulet,¶ Joaquin Sastre,‖ Santiago Quirce,‖‖ Dori Germolec,‖‖‖ Susan M. Tarlo,# Maria-Jesus Cruz,** Xavier Munoz,** Michael I. Luster,† and Berran Yucesoy†

*Division of Immunology, Allergy and Rheumatology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267; †CDC/National Institute for Occupational Safety and Health, Health Effects Laboratory Division, Morgantown, West Virginia 26505; ‡BRT-Burleson Research Technologies, Morrisville, North Carolina 27560; §Université de Montréal, Hôpital du Sacré-Coeur de Montréal, Montreal, Quebec, Canada H4J 1C5; ¶Université Laval, Hôpital Laval, Sainte-Foy, Québec, Canada G1Y 4G5; ‖Department of Allergy, Fundación Jiménez Díaz, Madrid, Spain 28040; ‖‖Department of Allergy, Hospital La Paz-IdiPAZ, Madrid, Spain 28046; ‖‖‖NIEHS/NIH, Research Triangle Park, North Carolina 27709; #University of Toronto, Toronto, Ontario, Canada M5G 1Z5; and **Hospitals Vall D'Hebron, Barcelona, Madrid, Spain 08035

[1]To whom correspondence should be addressed at Division of Immunology, Allergy and Rheumatology, University of Cincinnati Department of Internal Medicine, 3255 Eden Avenue, Suite 350, Cincinnati, OH 45267-0563. Fax: (513) 558-3799. E-mail: bernstdd@ucmail.uc.edu.

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Recently, a genome-wide association study (GWAS) conducted in Korean subjects identified four CTNNA3 (alpha-T catenin) single nucleotide polymorphisms (SNPs) (rs10762058, rs7088181, rs1786929, and rs4378283) associated with diisocyanate-induced occupational asthma (DA). The CTNNA3 gene codes for a cadherin involved in formation of stretch-resistant cell-cell adhesions. We conducted a candidate gene association study to replicate these findings in Caucasian workers. Genotyping was performed on DNA using a 5′ nuclease PCR assay collected from 410 diisocyanate-exposed and predominantly Canadian workers including 132 workers with DA confirmed by a specific inhalation challenge (DA+); 131 symptomatic workers in whom DA was excluded by a negative challenge (DA−); and 147 hexamethylene diisocyanate–exposed asymptomatic workers (AWs). As in the Korean study, highly linked CTNNA3 rs7088181 and rs10762058 SNPs (but not rs4378283 and rs1786929) were significantly associated with DA+ when compared with AWs but not in comparison with DA− workers ($p \leq 0.05$). After adjusting for potentially confounding variables of age, smoking status, and duration of exposure, minor allele homozygotes of rs7088181 and rs10762058 SNPs were at increased risk for DA compared with AWs (OR = 9.05 [95% CI: 1.69, 48.54] and OR = 6.82 [95% CI: 1.65, 28.24], respectively). In conclusion, we replicated results from the only reported GWAS study of DA demonstrating an association between two closely linked CTNNA3 gene SNPs and DA. These findings lend further support to the clinical relevance of these genotypes in predicting susceptibility to DA and the potential importance of catenins in the disease process.

Key Words: **diisocyanate asthma; CTNNA3; alpha-T catenin; occupational asthma.**

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Diisocyanates are reactive chemicals long recognized as leading causes of occupational asthma (OA) (Bakerly *et al*., 2008). Toluene diisocyanate (TDI), methylene diphenyl diisocyanate (MDI), and hexamethylene diisocyanate (HDI) are the most commonly used diisocyanates (Bernstein, 2011). Due to lack of diagnostic markers or screening tests, contribution of other potential occupational and environmental exposures, and variability of symptoms, the early diagnosis of diisocyanate-induced occupational asthma (DA) is challenging. Failure to recognize and diagnose affected workers in a timely fashion and to mitigate workplace exposure can result in impairment and disability due to severe asthma. Because there is no ideal diagnostic marker to identify new cases of DA early in its course, expensive worker surveillance programs are often implemented in large factories utilizing diisocyanates (Bernstein *et al*., 1993).

This unmet need has motivated the conduct of genetic association studies to better define genetic susceptibility for DA. Such studies are often limited by recruitment challenges due to the small numbers of workers diagnosed with DA. Nevertheless, a number of susceptibility and resistance loci have been identified in the HLA region (Bernstein, 2011), in immune response genes, including IL-4Rα, IL-13, and CD14 (Bernstein *et al*., 2006, 2011), and in antioxidant defense genes (Yucesoy *et al*., 2012).

Recently, the first and only genome-wide association study (GWAS) of DA was conducted in a group of 84 Korean workers with confirmed OA caused by respiratory sensitization to TDI (Kim *et al*., 2009). In this study, the CTNNA3 (alpha-T catenin) single nucleotide polymorphisms (SNPs) rs10762058, rs7088181, rs4378283, and another (rs1786929) identified by DNA sequencing were significantly associated with the TDI asthma phenotype compared with 264 unexposed healthy normal controls.

CTNNA3 is a key protein of the adherence junctional complex in epithelial cells and plays an important role in cellular adherence. The alpha-T-catenin protein is found primarily in the testes and heart. The E-cadherin/catenin (alpha-, beta-, and gamma-) complex has been investigated in cancer cells and found to play a mechanistic role in cell motility and even metastasis (Al Moustafa *et al*., 2002). In addition, CTNNA3 SNPs were found to be associated with late onset Alzheimer's disease (Blomqvist *et al*., 2004; Cellini *et al*., 2005). Although the putative role of this protein in the lungs or airways is unknown, antibodies to "self-antigens" including alpha-catenin, epidermal growth factor receptor, and activin A type 1 receptor have been identified in sera of asthmatic patients (Liu *et al*., 2012). In addition, de Boer *et al*. (2008) recently detected epithelial junctional adherence proteins E-cadherin, alpha-catenin, and beta-catenin in epithelial membranes of bronchial biopsies of asthmatic patients. A noteworthy finding was that alpha-catenin and E-cadherin expression were reduced in asthmatic versus nonasthmatic subjects, correlating inversely with numbers of eosinophils. These findings suggest that reduced expression of alpha-catenin and other junctional proteins could contribute to a dysfunctional airway epithelial barrier. Kim *et al*. (2009) reported that CTNNA3 mRNA expression was significantly lower in carriers of the minor haplotype of two SNPs (rs10762058 and rs7088181) compared with noncarriers. Although a functional role of CTNNA3 in the pathogenesis of TDI asthma remained to be determined, results for this study suggested that decreased expression of CTNNA3 might lead to increased susceptibility to TDI effects and contribute to disease development. Validation of results from GWAS studies in different ethnic populations is important in determining their clinical relevance. In this study, we sought to replicate results from the Korean GWAS in a Caucasian worker population.

MATERIALS AND METHODS

Study participants. Diisocyanate-exposed workers, referred for clinical evaluation of possible DA to occupational pulmonary disease clinics located in Canada (Sacre Coeur Hospital, Montreal; Laval Hospital, Sainte-Foy; University Health Network, Toronto) and Spain (Fundacion Jimenez Diaz, Madrid and Hospital Vall d'Hebron, Barcelona), were recruited for participation in this study. To confirm or exclude DA, controlled specific inhalation challenge testing (SIC) was performed in these clinics with the diisocyanates to which the worker was exposed according to published protocols (Malo *et al*., 1999; Sastre *et al*., 2003).

The study population included 410 diisocyanate-exposed workers with known exposure to TDI, MDI, or HDI and comprised 132 workers diagnosed with DA (DA+) confirmed by a positive SIC test, 131 workers reporting respiratory symptoms at work in whom DA was excluded by a negative SIC (DA−), and 147 HDI-exposed asymptomatic workers (AWs). A positive SIC response was defined as a fall in FEV_1 of at least 20% from prechallenge baseline following a controlled exposure to a diisocyanate chemical in a laboratory-based challenge chamber. In the 72 of the 131 DA− workers for whom another diagnosis could be determined, 41 (56.9%) were identified with non-occupational asthma, 12 (16.7%) with hyperventilation syndrome, 4 (5.55%) with rhinitis, 4 (5.55%) with occupational dermatitis, 2 (2.8%) with silicosis, and 1 (1.4%) was reported with OA attributable to styrene exposure. AWs were recruited from HDI-exposed spray painters living in Quebec and participating in a long-term surveillance study (Dragos *et al*., 2009). Data pertinent to demographics, smoking status, duration, and nature of chemical exposure were collected from all subjects. Atopic status was evaluated by skin prick testing to commercial aeroallergen extracts. Atopy was defined by a positive response with one or more aeroallergens defined by a 3-mm wheal ≥ saline control. Five to ten milliliters of whole blood was collected for genetic testing. All subjects gave informed consent, and the study protocol was approved and renewed annually by Institutional Review Boards at each participating institution and the National Institute for Occupational Safety and Health.

Gene selection and genotyping. The study population was tested for the CTNNA3 SNPs (rs7088181, rs10762058, rs1786929, and rs4378283) that were found to be significantly associated with DA phenotype in the aforementioned GWAS study (Kim *et al*., 2009). Genomic DNA was extracted from whole blood samples using the QIAamp blood kit (QIAGEN Inc., Chatsworth, CA). Genotyping was performed on genomic DNA using a 5´ nuclease PCR assay. Genotyping was performed using Applied Biosystems TaqMan Pre-Designed SNP Genotyping Assays (Foster City, CA). PCR amplification was performed in a volume of 25 µl containing 10 ng genomic DNA, 12.5 µl 2X TaqMan Universal Master Mix, 200nM of probe, and 900nM of primer. Cycling conditions were 50°C for 2 min and 95°C for 10 min, followed by 50 cycles at 92°C for 30 s and 60°C for 1 min. Amplification was performed using a StepOnePlus Real-Time PCR System (Applied Biosystems). Positive and negative controls were used within each run of PCR amplification. All samples with ambiguous results were repeated as was a random selection of 10% of all samples to ensure laboratory quality control.

Statistical analyses. Statistical analyses were generated using SAS/STAT software, Version 9.2 of the SAS system for Windows (SAS Institute, Cary, NC). These analyses made comparisons between AWs to both DA+ and DA− groups, as well as comparisons between DA+ and DA−. Group differences on demographic variables between DA+, DA−, and AW were assessed using chi-square statistics for categorical variables and ANOVAs with post hoc pairwise comparisons for continuous variables. Unadjusted tests of association between genetic variants and the outcome variable were assessed using Fisher's Exact Test. Three genetic models including 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 were assessed because the underlying genetic model is unknown. Unadjusted tests for additive model were assessed using the Cochran-Armitage Trend test. Logistic regression was utilized to model associations between genetic variants and the outcome variable while adjusting for confounding variables in each of the three genetic models described above. Confounding variables included age, smoking status, and duration of exposure. Sex and atopic status were not confounding variables in any model and were not included in the final analysis. Similar analyses were conducted only on French Canadians to test the possibility of bias due to population stratification. The distribution of the genotypes and the results of analyses were similar to those observed in combined sample of French-Canadian and Spanish, suggesting that population stratification was not a major confounder (data not shown). Because this was a candidate gene

TABLE 1
Demographics of the Study Groups

	AW	DA+	DA−
N	147	132	131
Sex, M/F	137/10	114/18	118/13
Age (mean years ± SD)	30.5 ± 7.6	40.9 ± 11.4	40.4 ± 10.3
Diisocyanate exposure (HDI/MDI/TDI)	147/0/0	60/27/45	98/22/11
Exposure duration (Mean months ± SD)	66.0 ± 27.0	135.3 ± 125.8	160.4 ± 137.0
Atopy (% positive)	57.4	55.8	57.2
Smoking Current/Ex/Never	53/29/65	22/49/61	43/42/44

Note. AW, asymptomatic diisocyanate-exposed controls; DA+, symptomatic exposed workers with SIC-confirmed diisocyanate asthma; DA−, symptomatic exposed workers with a SIC negative result.

study of specific known SNP associations with DA with the aim of replicating findings from a previous GWAS study, adjustment for multiple comparisons was not performed.

RESULTS

Subject Characteristics

Subject characteristics are shown in Table 1. Of the 410 workers evaluated, 369 (90%) were male. Eighty-six percent of subjects were of French-Canadian descent. The mean age was higher in the DA+ (40.9) and DA− (40.4) groups than in the AW group (30.5). Diisocyanate exposure was significantly different between the groups, with HDI being the predominant diisocyanate to which the majority of all workers (74%) were exposed. The frequency of atopy was similar between groups. The prevalence of current smoking was 17% in DA+, 33% in DA−, and 36% in AWs. The prevalence of current smoking was significantly less among DA+ workers versus AW controls ($p < 0.001$).

Genotype Frequencies and Multivariate Analysis

All allele frequencies in the control population were in Hardy-Weinberg equilibrium (data not shown). As shown in Table 2, the CTNNA3 rs7088181 and rs10762058 SNPs were significantly associated with the DA+ phenotype when compared with AWs ($p = 0.028$ and $p = 0.044$, respectively) but not in comparison with DA− workers ($p = 0.411$ and $p = 0.288$, respectively). No significant difference was observed in the distribution of genotypes between DA+ workers and either of the comparison groups for the CTNNA3 rs1786929 and CTNNA3 rs4378283 polymorphisms.

Logistic regression models were used to evaluate genotype associations with DA+ outcomes versus AWs adjusting for significant confounders including age, smoking status, and duration of exposure. Upon examination, only recessive models were statistically significant, the results of this model are presented in the Tables. Minor allele homozygotes of rs7088181 were at increased risk of DA with an odds ratio of 9.05 (95% CI: 1.69, 48.54) (Table 3). Interestingly, current smokers had significantly reduced risk of DA (OR = 0.31 [95% CI: 0.15, 0.64]). In addition, age and duration of diisocyanate exposure were independently associated with DA. Table 4 shows the same logistic regression analyses for the rs10762058 SNP. Workers homozygous for the minor allele were almost seven times more likely to have DA compared with AWs (OR = 6.82 [95% CI: 1.65, 28.24]). Because the sentinel findings of the rs7088181and rs10762058 associations were observed initially in TDI-exposed Korean workers with OA, we reanalyzed our data excluding TDI workers with DA and found the associations were maintained for MDI- and HDI-exposed subjects for rs7088181; a

TABLE 2
Distribution of CTNNA3 Genotype Frequencies Between the Groups

	DA+ ($n = 130$)	AWs ($n = 147$)	DA− ($n = 131$)	Fisher's exact *p* values	
Gene/SNP ID	*N* (%)	*N* (%)	*N* (%)	DA+ vs. AWs	DA+ vs. DA−
CTNNA3 rs7088181				0.028	0.411
AA	98 (75)	109 (74)	97 (74)		
AG	25 (18)	36 (24)	29 (22)		
GG	9 (7)	2 (1)	5 (4)		
CTNNA3 rs10762058				0.044	0.288
CC	95 (72)	102 (70)	92 (70)		
CG	27 (20)	41 (28)	34 (26)		
GG	10 (8)	3 (2)	5 (4)		
CTNNA3 rs1786929				0.211	1.000
TT	60 (45)	63 (43)	65 (50)		
CT	59 (45)	62 (42)	53 (40)		
CC	13 (10)	22 (15)	13 (10)		
CTNNA3 rs4378283				0.473	1.000
CC	124 (94)	131 (89)	122 (93)		
CT	7 (5)	16 (11)	8 (6)		
TT	1 (1)	0 (0)	1 (1)		

Note. AW, asymptomatic diisocyanate-exposed controls; DA+, symptomatic exposed workers with SIC-confirmed diisocyanate asthma; DA−, symptomatic exposed workers with a SIC negative result.

TABLE 3
Logistic Regression Model for CTNNA3 rs7088181 (DA+ vs. AW Comparator Group)

Effect(recessive model)	Odds ratio(95% confidence intervals)	*p* Value[a]
CTNNA3 rs7088181 (1.1 + 1.2) vs 2.2[b]	9.05 (1.69, 48.54)	0.01
Age (years)	1.10 (1.06, 1.14)	< 0.0001
Smoking (current vs. never)	0.31 (0.15, 0.64)	0.0017
Exposure duration (months)	1.01 (1.00, 1.01)	0.0019

Note. AW, asymptomatic diisocyanate-exposed controls; DA+, symptomatic exposed workers with SIC-confirmed diisocyanate asthma.
[a]The results were adjusted for age, smoking, and exposure duration.
[b]Genotypes: 1.1, homozygous for the major allele; 2.2, homozygous for the minor allele; 1.2, heterozygous.

TABLE 4
Logistic Regression Model for CTNNA3 rs10762058 (DA+ vs. AW Comparator Group)

Effect(recessive model)	**Odds ratio (95% confidence intervals)**	***p* Value[a]**
CTNNA3 rs10762058 (1.1 + 1.2) vs 2.2[b]	6.82 (1.65, 28.24)	0.008
Age (years)	1.10 (1.06, 1.14)	< 0.0001
Smoking (current vs. never)	0.32 (0.15, 0.67)	0.0024
Exposure duration (months)	1.01 (1.00, 1.01)	0.0020

Note. AW, asymptomatic diisocyanate-exposed controls; DA+, symptomatic exposed workers with SIC-confirmed diisocyanate asthma.
[a]The results were adjusted for age, smoking, and exposure duration.
[b]Genotypes: 1.1, homozygous for the major allele; 2.2, homozygous for the minor allele; 1.2, heterozygous.

nonsignificant trend was maintained for the rs10762058 SNP ($p = 0.08$).

DISCUSSION

In this study, we sought to replicate the results of the only GWAS study reported for DA, which was carried out in a Korean population of TDI-exposed workers, using a comparison group of nonexposed healthy normal controls. Kim *et al.* (2009) identified three SNPs in the CTNNA3 gene encoding alpha-T-catenin on chromosome 10q21 that remained significantly associated with confirmed DA after adjusting for sex and age as confounders (rs7088181 [$p = 1.4 \times 10^{-5}$]; rs10762058 [$p = 5.8 \times 10^{-6}$]; and rs4378283 [$p = 2 \times 10^{-5}$]). Two of the CTNNA3 SNPs, rs10762058 and rs7088181, are known to be in strong linkage disequilibrium. In the Korean study, DNA sequencing studies identified a fourth CTNNA3 SNP, rs1786929, associated with DA. In our study of Caucasian workers of European descent, we evaluated possible associations of these four CTNNA3 SNPs with DA confirmed by SIC testing in comparison with exposed symptomatic workers with a negative SIC test (DA–) and asymptomatic diisocyanate-exposed workers (AWs). Analyses using unadjusted frequencies, after adjusting for multiple significant covariates, were able to replicate the associations between DA+ and AWs for two of the CTNNA3 SNPs (rs7088181 and rs10762058) under a recessive logistic model. The association of both of these SNPs with DA could be explained by their high linkage at the CTNNA3 locus (D´ > 0.9) (Kim *et al.*, 2009). It is noteworthy that significant associations were not detected in the other comparison group, symptomatic workers in whom OA had been excluded (i.e., DA–). This result is consistent with most of the prior candidate gene studies of DA, demonstrating significant DA associations with asymptomatic subject comparator groups (Bernstein, 2011; Piirilä *et al.*, 2001).

In addition to genotype predictors of DA, we also considered several potential confounders in the final logistic regression models including age at diagnosis, duration of diisocyanate exposure, and current smoking status (Tables 3 and 4). These confounding factors were also independently associated with DA in all logistic models. Mapp *et al.* (2000) first recognized the relatively low frequency of smoking in workers with DA, although the modifying effect of smoking on DA has not been observed in other epidemiologic studies of DA (Mapp *et al.*, 1988). The unexplained association of DA with nonsmoking status in this study must be interpreted cautiously. This was a case control study and subject to inherent design limitations including recall and group selection bias. Therefore, this finding requires confirmation in future longitudinal studies.

Genetic studies of workers with OA present numerous challenges. The low incidence of OA limits the ability of investigators to recruit large and multiple study populations to replicate results from association studies. Nonetheless, the capacity to identify causation and to precisely define a specific OA phenotype such as DA by SIC testing with the causative agent offers an advantage over genetic association studies of non-OA, which often must rely on intermediate phenotypes (e.g., airway hyperresponsiveness, total IgE). In this respect, a number of genetic studies in workers with DA have revealed strong associations with disease. The HLA region has been studied extensively with respect to disease susceptibility due to its role in antigen recognition/presentation (Bernstein, 2011; Bernstein *et al.*, 2011). In particular, the HLA DQA1*0104 and DQB1*0503 alleles were found to be associated with susceptibility to DA, whereas the DQB1*0501 allele and the DQA1*0101-DQB1*0501-DR1 haplotype were considered protective (Bignon *et al.*, 1994; Mapp *et al.*, 2000). We previously investigated associations between SNPs in immune response genes (IL-4Rα, IL-13, and CD14) and DA. The results demonstrated a gene-environment interaction between HDI exposure and combinatorial genotypes of these SNPs (Bernstein *et al.*, 2006). Because diisocyanates are known to cause oxidative injury in respiratory epithelial cells, the variations within antioxidant defense genes have also been examined in relation to DA. These studies identified a number of variants to be associated with DA susceptibility including GSTM1 deletion, GSTP1 Ile105Val, and the NAT1 (N-acetyltransferase) slow acetylator genotype (Piirilä *et al.*, 2001; Wikman *et al.*, 2002). We recently reported an association between DA and variants in SOD2, GST, and EPHX1 genes, either individually or in combination (Yucesoy *et al.*, 2012).

The major strengths of this study include a well-characterized phenotype and examination of genetic associations while adjusting for potential confounding factors such as atopy, smoking status, exposure duration, and specific diisocyanate exposure. The major limitations include the small sample size due to rarity of DA and the fact that the controls were younger and had less exposure to diisocyanates than cases. The young age of the controls was unavoidable due to the difficulty in recruiting age-matched workplace controls and may be problematic in terms of detection of age-related associations. However, the lack of association between the SNPs and DA− workers whose age closely matched that of the DA+ group suggests that the association seen between DA+ and AW workers might be specific for DA. The results were not corrected for multiple comparisons because our study was hypothesis driven. Instead, we reported all tests that reached the 0.05 level of significance.

In summary, we have replicated results from a GWAS study demonstrating an association between two linked SNPs of the CTNNA3 gene and DA. These findings suggest that genetically determined reduced expression of alpha-T-catenin might influence cellular adherence in the airways and could play a role in the pathogenesis of DA.

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REFERENCES

Al Moustafa, A. E., Yen, L., Benlimame, N., and Alaoui-Jamali, M. A. (2002). Regulation of E-cadherin/catenin complex patterns by epidermal growth factor receptor modulation in human lung cancer cells. *Lung Cancer* **37,** 49–56.

Bakerly, N. D., Moore, V. C., Vellore, A. D., Jaakkola, M. S., Robertson, A. S., and Burge, P. S. (2008). Fifteen-year trends in occupational asthma: Data from the Shield surveillance scheme. *Occup. Med. (Lond).* **58,** 169–174.

Bernstein, D. I., Korbee, L., Stauder, T., Bernstein, J. A., Scinto, J., Herd, Z. L., and Bernstein, I. L. (1993). The low prevalence of occupational asthma and antibody-dependent sensitization to diphenylmethane diisocyanate in a plant engineered for minimal exposure to diisocyanates. *J. Allergy Clin. Immunol.* **92,** 387–396.

Bernstein, D. I., Wang, N., Campo, P., Chakraborty, R., Smith, A., Cartier, A., Boulet, L. P., Malo, J. L., Yucesoy, B., Luster, M., *et al.* (2006). Diisocyanate asthma and gene-environment interactions with IL4RA, CD-14, and IL-13 genes. *Ann. Allergy Asthma Immunol.* **97,** 800–806.

Bernstein, D. I. (2011). Genetics of occupational asthma. *Curr. Opin. Allergy Clin. Immunol.* **11,** 86–89.

Bernstein, D. I., Kissling, G. E., Khurana Hershey, G., Yucesoy, B., Johnson, V. J., Cartier, A., Gautrin, D., Sastre, J., Boulet, L. P., Malo, J. L., *et al.* (2011). Hexamethylene diisocyanate asthma is associated with genetic polymorphisms of CD14, IL-13, and IL-4 receptor α. *J. Allergy Clin. Immunol.* **128,** 418–420.

Bignon, J. S., Aron, Y., Ju, L. Y., Kopferschmitt, M. C., Garnier, R., Mapp, C., Fabbri, L. M., Pauli, G., Lockhart, A., and Charron, D. (1994). HLA class II alleles in isocyanate-induced asthma. *Am. J. Respir. Crit. Care Med.* **149,** 71–75.

Blomqvist, M. E., Andreasen, N., Bogdanovic, N., Blennow, K., Brookes, A. J., and Prince, J. A. (2004). Genetic variation in CTNNA3 encoding alpha-3 catenin and Alzheimer's disease. *Neurosci. Lett.* **358,** 220–222.

Cellini, E., Bagnoli, S., Tedde, A., Nacmias, B., Piacentini, S., and Sorbi, S. (2005). Insulin degrading enzyme and alpha-3 catenin polymorphisms in Italian patients with Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* **19,** 246–247.

de Boer, W. I., Sharma, H. S., Baelemans, S. M., Hoogsteden, H. C., Lambrecht, B. N., and Braunstahl, G. J. (2008). Altered expression of epithelial junctional proteins in atopic asthma: Possible role in inflammation. *Can. J. Physiol. Pharmacol.* **86,** 105–112.

Dragos, M., Jones, M., Malo, J. L., Ghezzo, H., and Gautrin, D. (2009). Specific antibodies to diisocyanate and work-related respiratory symptoms in apprentice car-painters. *Occup. Environ. Med.* **66,** 227–234.

Kim, S. H., Cho, B. Y., Park, C. S., Shin, E. S., Cho, E. Y., Yang, E. M., Kim, C. W., Hong, C. S., Lee, J. E., and Park, H. S. (2009). Alpha-T-catenin (CTNNA3) gene was identified as a risk variant for toluene diisocyanate-induced asthma by genome-wide association analysis. *Clin. Exp. Allergy* **39,** 203–212.

Liu, M., Subramanian, V., Christie, C., Castro, M., and Mohanakumar, T. (2012). Immune responses to self-antigens in asthma patients: Clinical and immunopathological implications. *Hum. Immunol.* **73,** 511–516.

Malo, J. L., Ghezzo, H., and Elie, R. (1999). Occupational asthma caused by isocyanates: Patterns of asthmatic reactions to increasing day-to-day doses. *Am. J. Respir. Crit. Care Med.* **159,** 1879–1883.

Sastre, J., Fernández-Nieto, M., Novalbos, A., De Las Heras, M., Cuesta, J., and Quirce, S. (2003). Need for monitoring nonspecific bronchial hyperresponsiveness before and after isocyanate inhalation challenge. *Chest* **123,** 1276–1279.

Mapp, C. E., Boschetto, P., Dal Vecchio, L., Maestrelli, P., and Fabbri, L. M. (1988). Occupational asthma due to isocyanates. *Eur. Respir. J.* **1,** 273–279.

Mapp, C. E., Beghè, B., Balboni, A., Zamorani, G., Padoan, M., Jovine, L., Baricordi, O. R., and Fabbri, L. M. (2000). Association between HLA genes and susceptibility to toluene diisocyanate-induced asthma. *Clin. Exp. Allergy* **30,** 651–656.

Piirilä, P., Wikman, H., Luukkonen, R., Kääriä, K., Rosenberg, C., Nordman, H., Norppa, H., Vainio, H., and Hirvonen, A. (2001). Glutathione S-transferase genotypes and allergic responses to diisocyanate exposure. *Pharmacogenetics* **11,** 437–445.

Wikman, H., Piirilä, P., Rosenberg, C., Luukkonen, R., Kääriä, K., Nordman, H., Norppa, H., Vainio, H., and Hirvonen, A. (2002). N-Acetyltransferase genotypes as modifiers of diisocyanate exposure-associated asthma risk. *Pharmacogenetics* **12,** 227–233.

Yucesoy, B., Johnson, V. J., Lummus, Z. L., Kissling, G. E., Fluharty, K., Gautrin, D., Malo, J. L., Cartier, A., Boulet, L. P., Sastre, J., *et al.* (2012). Genetic variants in antioxidant genes are associated with diisocyanate-induced asthma. *Toxicol. Sci.* **129,** 166–173.