N-Acetyltransferase 2 Genotypes Are Associated With Diisocyanate-Induced Asthma

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Objective: To investigate whether genetic variants of N-acetyltransferase (*NAT*) genes are associated with diisocyanate asthma (DA). **Methods:** The study population consisted of 354 diisocyanate-exposed workers. Genotyping was performed using a 5′-nuclease polymerase chain reaction assay. **Results:** The *NAT2* rs2410556 and *NAT2* rs4271002 variants were significantly associated with DA in the univariate analysis. In the first logistic regression model comparing DA+ and asymptomatic worker groups, the rs2410556 ($P = 0.004$) and rs4271002 ($P < 0.001$) single nucleotide polymorphisms and the genotype combination, *NAT2* rs4271002∗*NAT1* rs11777998, showed associations with DA risk ($P = 0.014$). In the second model comparing DA+ and DA− groups, *NAT2* rs4271002 variant and the combined genotype, *NAT1* rs8190845∗*NAT2* rs13277605, were significantly associated with DA risk ($P = 0.022$, $P = 0.036$, respectively). **Conclusions:** These findings suggest that variations in the *NAT2* gene and their interactions contribute to DA susceptibility.

Occupational asthma (OA) is characterized by variable airflow obstruction, airway hyperresponsiveness, and/or inflammation caused by workplace exposure to certain substances and may account for 10% to 25% of all adult cases of asthma.[1,2] Diisocyanates, low-molecular weight reactive chemicals used in industry to generate polyurethane, are a leading cause of OA in industrialized countries. Toluene diisocyanate (TDI), 4,4′-diphenylmethane diisocyanate (MDI), and hexamethylene diisocyanate (HDI) are the most commonly used monomers in industry. Centers for Disease Control and Prevention (CDC) estimates that over 280,000 workers are exposed to diisocyanates in the workplace and 5% to 15% of them with chronic exposure develop OA.[3–6]

Current evidence suggests that the pathophysiology of diisocyanate asthma (DA) involves chronic airway inflammation and oxidative stress in the lungs. Following inhalation of diisocyanates, reactive oxygen and nitrogen species generated by activated inflammatory and bronchial epithelial cells induce a respiratory burst and result in tissue injury.[7–9] In vivo and in vitro studies have shown that diisocyanates alter thiol-redox homeostasis of airway epithelium.[10,11] Marczynski et al[12] showed the formation of H_2O_2 in white blood cells of subjects after diisocyanate exposure. Another study reported altered expression of proteins involved in oxidant/antioxidant-mediated airway inflammation in MDI-asthma patients.[13] Human serum albumin-conjugated TDI was found to induce oxidative stress in bronchial epithelial cells.[14] In a mouse model, expression of oxidative stress and thiol-redox balance-related genes was increased following polymeric HDI exposure.[15] These findings suggest that oxidative stress is a major contributor to persistent airway inflammation and tissue damage in DA. A number of enzymatic antioxidants, including glutathione S-transferases (*GST*s), manganese superoxide dismutase (*SOD2*), and microsomal epoxide hydrolase (*EPHX1*), play a major protective role in redox balance in the lung as well as help regulate oxidant-induced inflammatory responses. In support of this mechanism, we recently reported that genetic variations in *SOD2*, *GST*, and *EPHX1* genes and their interactions contribute to DA susceptibility.[16]

In the present study, we evaluated associations between DA and gene variants of N-acetyltransferase (NAT) enzymes involved in the activation/inactivation of numerous xenobiotics. The *NAT1* and *NAT2* genes are both located on chromosome 8 (8p21.3–23.1 and 8p21.3–23.1 and 8p22, respectively) and catalyze N-acetylation and O-acetylation of aromatic and heterocyclic amines.[17,18] They are also involved in the deactivation of pro-inflammatory cysteinyl leukotrienes which are potent mediators of airway narrowing.[19] Both *NAT1* and *NAT2* are expressed in the airway epithelium and show wide interindividual variation.[20,21] NATs are also known to be involved in the deactivation of aromatic amines that can be formed from diisocyanates in aqueous environments.[22,23] Because oxidative stress is an important early event in diisocyanate-induced respiratory damage, genetic modification of the enzymatic activity of NATs can directly influence the expression of disease. The aim of this study was to identify NAT single nucleotide polymorphisms (SNP) that could influence genetic susceptibility to DA.

METHODS

Study Participants

The initial study population consisted of 411 diisocyanate (HDI, MDI, and TDI)-exposed workers. This population was composed of three distinct phenotypes including: (1) 132 workers diagnosed with DA (DA+) based on a positive specific inhalation challenge (SIC) test; (2) 131 workers reporting respiratory symptoms at work in whom DA was excluded based on a negative SIC (DA−); and (3) 148 HDI-exposed asymptomatic worker (AW) controls. The main study analyses were conducted on only Caucasian French-Canadian workers ($n = 354$) to avoid the possibility of bias because of population stratification[24]; supplemental analyses were also conducted on the entire sample, as described below.

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Symptomatic subjects were recruited from 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). The subjects underwent SIC with the appropriate work-relevant diisocyanate chemicals according to previously described protocols.[25,26] Patients were classified as DA+ or DA− based on their positive and negative responses to diisocyanate SIC, respectively. A decrease in FEV_1 of at least 20% from pre-challenge baseline during the early and/or late asthmatic response was defined as a positive SIC test. AW controls were recruited in Quebec, Canada, from HDI-exposed painters. Data regarding age, sex, ethnicity, smoking status, duration of exposure, and respiratory symptoms 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. Whole blood was collected for genetic testing. All subjects gave informed consent, and the study protocol is approved and renewed annually by Institutional Review Boards of each participating institution.

Gene Selection and Genotyping

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 polymerase chain reaction (PCR) assay. Primers and probes were designed, using the Assay-by-Design™ service from Applied Biosystems (Foster City, CA). The QuickSNP version 1.1 was used to select a total of 18 tagSNPs within the *NAT1* and *NAT2* genes based on linkage disequilibrium (LD) structure (SNPs with an r^2 greater than 0.8 were represented by one tagSNP) and minor allele frequency greater than 0.05 using the HapMap data (Caucasians).[27] Positive and negative controls were used within each run of PCR amplification. All samples with ambiguous results were repeated as were a random selection of 10% of all samples to ensure laboratory quality control.

Statistical Analyses

The primary analysis was restricted to Caucasian French-Canadians to minimize bias because of population stratification. The numbers of subjects recruited from other non-Caucasian French-Canadian ($n = 31$) and Spanish ($n = 26$) populations was too small to independently support statistical model development. The same final logistic models, however, were fit to the entire sample and results of those are included as supplementary data. Because of the low prevalence of some of the minor alleles, a dominant model for each SNP was used for the statistical analyses, that is, SNP variables were coded as 0 if the subject was homozygous for the major allele and 1 if the subject was heterozygous or homozygous for the minor allele. Potential associations between each SNP and DA, adjusting for the demographic variables of age, smoking status, type of exposure (for the DA+ vs DA− analysis only), and length of exposure, were first tested in a series of logistic regression models that investigated one SNP at a time. The sample size did not support including all possible two-way interactions between pairs of SNPs in a logistic regression model; therefore, two-way interactions were screened in a second series of logistic regressions that contained the demographic variables listed above and a pair of dominant-model-coded SNPs with their two-way interaction term; every possible pair, in turn, was considered. SNP main effects and two-way interactions found significant at the 0.05 level in these two series of logistic regression models were put into a final logistic regression model that also contained the demographic variables listed above. Two such final models were estimated: (1) a comparison of DA+ and AW controls, and (2) a comparison of DA+ and DA− symptomatic groups. The model for the DA+ and DA− symptomatic groups was over-parameterized when all terms selected as described above were included, so backward elimination at significance level 0.05 was applied to yield the final model. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). SNAP was used to find proxy SNPs within 500 kb based on LD and physical distance.[28] RegulomeDB was used to annotate SNPs with known and predicted regulatory elements.[29]

RESULTS

The demographic characteristics of the Caucasian French-Canadians included in the statistical analyses are described in Table 1. Mean age was higher in the DA+ and DA− groups than AW controls (42.3, 40.3 vs 30.3 years; overall $P < 0.001$). Type of diisocyanate exposure (HDI vs MDI vs TDI) differed significantly between the groups (overall $P < 0.001$). Although the duration of work exposure was similar between the DA+ and DA− worker groups (144.6 vs 164.9 months, $P = 0.297$), the AW controls had less exposure to isocyanates than both groups (65.8 months, $P < 0.001$). The frequency of atopy was similar in all three groups (overall $P = 0.872$). The prevalence of smoking was significantly different between DA+ and AW controls (overall $P < 0.001$). The overall type and the severity of the respiratory symptoms (eg, cough, wheezing, shortness of breath, tightness in chest) were similar in symptomatic groups. The allele frequencies in the control population were similar to those determined in other studies involving Caucasian populations and were in Hardy-Weinberg equilibrium (data not shown). The demographic characteristics of the entire study sample are given in Supplementary Table 1 (http://links.lww.com/JOM/A226).

Table 2 shows the distribution of genotype frequencies between the study groups (French-Canadians) and the *P* values represent the comparison of the proportions of genotypes between two groups under a dominant genetic model. The univariate analyses for dominant-model SNPs for the HDI-exposed DA+, DA−, and AW control groups (French-Canadians) are presented in Supplementary Table 2 (http://links.lww.com/JOM/A227). The *NAT2* rs2410556 and rs4271002 SNPs were the only candidate SNPs that were individually significantly associated with the DA diagnosis. The distribution of *NAT2* rs2410556 and rs4271002 genotypes was significantly different in DA+ workers compared with DA− group ($P = 0.004$, $P = 0.001$, respectively) and AW controls (both at $P < 0.001$). The univariate analyses for dominant-model SNPs for the HDI-exposed DA+ and AW control groups (entire sample) are presented in Supplementary Table 3A (http://links.lww.com/JOM/A228) and for the DA+ and DA− (entire sample) in Supplementary Table 3B (http://links.lww.com/JOM/A228).

A logistic regression analysis was then conducted for each SNP separately (under a dominant genetic model) adjusting for age, smoking, type of exposure, and length of exposure (Supplementary Tables 4A and 4B, http://links.lww.com/JOM/A229). To identify potential interactions between SNPs, another logistic regression analysis was performed on all SNP–SNP combinations, adjusting for demographic variables (Supplementary Tables 5A and 5B, http://links.lww.com/JOM/A230). Tables 3 and 4 present final logistic regression models, including demographic variables, all significant SNPs, and all potential significant interactions identified in aforementioned logistic regression analyses. SNPs were dichotomized as carriers of the minor allele (homozygote or heterozygote) versus major allele homozygotes. For the interaction terms, the odds ratio (OR) represents the odds of DA+ for carriers of at least one minor allele at both SNPs versus the odds of DA+ for any other genotype combination. The results of analyses on the larger sample that included subjects from Spain and non-Caucasian

TABLE 1. Characteristics of Study Participants

	DA+	DA−	AWs	DA+ vs DA− *P*	DA+ vs AWs *P*	Overall *P*
N	95	117	142			
Sex, M/F	84/11	106/11	132/10	0.605	0.228	0.484
Age at diagnosis ± SE	42.3 ± 1.2	40.3 ± 0.9	30.3 ± 0.6	0.177	<0.001	<0.001
5th percentile	23.5	23.5	24.2			
25th percentile	32.5	32.7	25.5			
Median	41.3	40.9	27.6			
75th percentile	52.1	47.2	30.7			
95th percentile	62.2	57.8	47.0			
Range	21.2–65.0	18.8–64.0	23.4–60.6			
Diisocyanate exposure (HDI/MDI/TDI)	53/22/20	93/19/5	142/0/0	<0.001	<0.001	<0.001
Duration of exposure, mo ± SE	144.6 ± 14.4	164.9 ± 13.0	65.8 ± 2.3	0.297	<0.001	<0.001
5th percentile	4	5	4.5			
25th percentile	31	42	57			
Median	96	123	72			
75th percentile	216	264	83			
95th percentile	456	408	102			
Range	1–540	1–660	3–113			
Skin prick test						
Positive/negative	56/36	63/47	76/54	0.605	0.719	0.872
Smoker (current/ex/never)	16/36/43	39/38/38	52/27/63	0.017	<0.001	0.001
Pack-yrs ± SE	11.9 ± 1.6	11.3 ± 1.3	5.8 ± 0.7	0.746	<0.001	<0.001
5th percentile	0	0	0			
25th percentile	0	0	0			
Median	1.8	6.0	1.1			
75th percentile	25	19	10			
95th percentile	40	33	26			
Range	0–52	0–85	0–45			

AW, asymptomatic worker; DA, diisocyanate asthma; HDI, hexamethylene diisocyanate; MDI, methylene diphenyl diisocyanate; TDI, toluene diisocyanate.

French-Canadians are shown in Supplementary Tables 6 (http://links.lww.com/JOM/A231) and 7 (http://links.lww.com/JOM/A232).

The first logistic regression model included DA+ and AW groups and adjusted for age, smoking status, and length of exposure (Table 3). Only HDI-induced DA+ cases were taken into consideration since AW controls were exposed only to HDI. The *NAT2* rs2410556 and rs4271002 SNPs were associated with an increased risk of DA with an OR of 4.79 (95% confidence interval [CI], 1.64 to 14.01, $P = 0.004$) and 24.2 (95% CI, 5.68 to 103.5, $P < 0.001$), respectively. In addition, the combined genotype of *NAT2* rs4271002∗*NAT1* rs11777998 was associated with a decreased risk of DA with an OR of 0.05 (95% CI, <0.01 to 0.54, $P = 0.014$). For this genotype combination, 12 of 51 HDI-exposed DA+ subjects (23.5%) carried minor alleles at both SNPs, whereas 23 of 141 AW subjects (16.3%) carried minor alleles at both SNPs; 28 of 51 HDI-exposed DA+ subjects (54.9%) carried only one minor allele, whereas 22 of 141 HDI-exposed AW subjects (15.6%) carried only one minor allele.

The second model included DA+ and DA− groups and adjusted for age, smoking status, type of diisocyanate exposure (HDI vs MDI or TDI) and length of exposure (Table 4). The carriage of the minor allele for the *NAT2* rs4271002 SNP was associated with an increased risk of DA with an OR of 2.44 (95% CI, 1.14 to 5.24, $P = 0.022$). The copresence of variant genotypes of the *NAT1* rs8190845 and *NAT2* rs13277605 SNPs was associated with an increased risk of DA with an OR of 8.39 (95% CI, 1.14 to 61.7, $P = 0.036$). For this genotype combination, 20 of 92 DA+ subjects (21.7%) carried minor alleles at both SNPs, whereas 10 of 108 DA− subjects (9.2%) carried minor alleles at both SNPs; 53 of 92 DA+ subjects (57.6%) carried only one minor allele, whereas 73 of 108 DA− subjects (67.6%) carried only one minor allele at the two SNPs.

The five significant SNPs identified from data analysis were used as inputs to the SNAP SNP Annotation and Proxy Search tools to update SNP IDs according to dbSNP135 and to find additional SNPs in LD (using an r^2 of 1). This led to the identification of an additional five correlated SNPs using data from the International HapMap Project (release 22). The total set of 10 SNPs was then used as inputs to the RegulomeDB web source, 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.[29] Six SNPs (rs13277605, rs2410556, rs11777998, rs1495749, rs13270034, and rs8190845) showed minimal binding evidence (RegulomeDB scores 5 and 6). We were unable to find information pertaining to the possible functional role for the other significant (rs4271002) and correlated (rs17642674, rs12548816, and rs8190870) SNPs.

DISCUSSION

The present candidate gene association study showed significant associations between DA and *NAT2* variants. Two *NAT2* variants, rs2410556 and rs4271002, were significantly associated with DA in both univariate and multivariable analyses when evaluated against two control comparator groups (ie, AW and DA− groups). In addition, the combined genotypes of rs4271002∗rs11777998 and rs8190845∗rs13277605 showed associations with DA. Altered risk related to these variant combinations is context-dependent, and suggests that some SNPs display significant association when considered as part of an SNP-covariate or SNP–SNP interaction. These results suggest previously unrecognized associations of *NAT* genotypes with the symptomatic workers population. The functional consequence and the role of these SNPs in DA process have not been previously investigated.

A number of studies have reported associations between slow acetylation *NAT2* genotypes and the risk of bronchial asthma.[30–33]

TABLE 2. Distribution of Genotype Frequencies Between the Groups in a Dominant Genetic Model (French-Canadians Only)

Gene/SNP ID		DA+ ($n=95$) *N* (%)	DA− ($n=116$) *N* (%)	AW ($n=142$) *N* (%)	Chi-Square *P* DA+ vs DA−	Chi-Square *P* DA+ vs AW
			NAT1			
rs11777998	GG	70 (74.5)	93 (80.2)	106 (74.6)	0.324	0.975
	GC or CC	24 (25.5)	23 (19.8)	36 (25.4)		
rs13253389	GG	42 (44.2)	52 (45.2)	52 (36.6)	0.884	0.242
	GA or AA	53 (55.8)	63 (54.8)	90 (63.4)		
rs4298522	TT	46 (48.4)	42 (36.2)	62 (44.0)	0.073	0.501
	TA or AA	49 (51.6)	74 (63.8)	79 (56.0)		
rs4921580	CC	75 (78.9)	90 (78.3)	104 (73.2)	0.904	0.316
	CG or GG	20 (21.1)	25 (21.7)	38 (26.8)		
rs4921880	AA	53 (55.8)	76 (65.5)	83 (58.4)	0.149	0.685
	AT or TT	42 (44.2)	40 (34.5)	59 (41.6)		
rs7003890	TT	31 (32.6)	34 (29.1)	47 (33.1)	0.575	0.940
	TC or CC	64 (67.4)	83 (70.9)	95 (66.9)		
rs7017402	GG	73 (76.8)	90 (77.6)	105 (73.9)	0.898	0.613
	AG or AA	22 (23.2)	26 (22.4)	37 (26.2)		
rs8190837	AA	80 (84.2)	106 (91.4)	126 (88.7)	0.109	0.312
	AG or GG	15 (15.8)	10 (8.6)	16 (11.3)		
rs8190845	GG	73 (76.8)	92 (78.6)	111 (78.7)	0.755	0.732
	AG or GG	22 (23.2)	25 (21.4)	30 (21.3)		
rs9325827	TT	68 (72.3)	82 (70.7)	99 (69.7)	0.792	0.665
	CT or CC	26 (27.7)	34 (29.3)	43 (30.3)		
			NAT2			
rs13277605	GG	21 (22.6)	38 (33.6)	41 (29.3)	0.081	0.257
	GT or TT	72 (77.4)	75 (66.4)	99 (70.7)		
rs1801280	TT	26 (27.4)	25 (21.6)	34 (23.9)	0.326	0.552
	TC or CC	69 (72.6)	91 (78.4)	108 (76.1)		
rs1961456	AA	53 (56.4)	57 (49.6)	88 (62.0)	0.326	0.391
	GA or GG	41 (43.6)	58 (50.4)	54 (38.0)		
rs2410556	TT	24 (25.5)	51 (45.1)	100 (70.9)	0.004	<0.001
	CT or CC	70 (74.5)	62 (54.9)	41 (29.1)		
rs4271002	GG	27 (28.7)	58 (50.9)	110 (77.5)	0.001	<0.001
	GC or CC	67 (71.3)	56 (49.1)	32 (22.5)		
rs4646246	AA	69 (72.6)	77 (66.4)	113 (80.1)	0.328	0.178
	AG or GG	26 (27.4)	39 (33.6)	28 (19.9)		
rs1799930	GG	55 (58.5)	58 (49.6)	77 (54.2)	0.196	0.516
	GA or AA	39 (41.5)	59 (50.4)	65 (45.8)		
rs1799931	GG	92 (96.8)	109 (94.0)	139 (97.9)	0.517*	0.686*
	GA or AA	3 (3.2)	7 (6.0)	3 (2.1)		

AW, asymptomatic worker; DA, diisocyanate asthma; NAT, N-acetyltransferase.
*Fisher's exact test *P* value.

A recent meta-analysis showed that slow acetylator *NAT2* genotypes might increase asthma risk among Caucasians (OR 2.20; 95% CI, 1.31 to 3.72).[34] To our knowledge, there have been two other studies examining the role of *NAT* variants in DA. Earlier Berode and Savolainen[35] reported that *NAT2* slow acetylator individuals, exposed to common diisocyanate monomers at work, are more susceptible to asthma. Later, they confirmed their finding in a larger study, and showed that *NAT2* slow acetylation could be a surrogate

TABLE 3. Logistic Regression Model: DA+ ($n=51$) vs AW ($n=141$) in HDI-Exposed Subjects

Model term	Estimate, β	SE	Chi-Square	OR (95% CI)	*P**
Intercept	−4.6750	1.1741	15.85		<0.001
Age, yrs	0.0774	0.0290	7.12	1.08 (1.02, 1.14)	0.008
Smoking					
Ex-smoker vs never smoked	0.4590	0.4290	1.14	0.69 (0.29, 2.48)	0.285
Current smoker vs never smoked	−1.2834	0.4955	6.71	0.12 (0.03, 0.56)	0.010
Months of exposure	0.0194	0.0058	11.14	1.02 (1.01, 1.03)	<0.001
NAT2 rs2410556	0.7836	0.2736	8.21	4.79 (1.64, 14.01)	0.004
NAT2 rs4271002	1.5939	0.3702	18.53	24.2 (5.68, 103.5)	<0.001
NAT1 rs11777998	0.4178	0.4602	0.8241	2.31 (0.38, 14.00)	0.364
NAT2 rs4271002∗*NAT1* rs11777998	−3.0462	1.2447	5.99	0.05 (<0.01, 0.54)[a]	0.014

AW, asymptomatic worker; CI, confidence interval; DA, diisocyanate asthma; HDI, hexamethylene diisocyanate; NAT, N-acetyltransferase; OR, odds ratio.
*Adjusted for age, smoking status, and length of exposure.
[a]Odds of DA + when minor alleles are present at both SNPs vs when only major alleles are present at one or both SNPs. Goodness of fit: chi-square = 5.71 with 8 *df*, $P=0.679$.

TABLE 4. Logistic Regression Model for Significant Variations, DA+ ($n = 92$) vs DA− ($n = 108$)

Model Term	Estimate, β	SE	Chi-Square	OR (95% CI)	P^*
Intercept	−2.5436	0.8550	8.85		0.003
Age, yrs	0.0450	0.0203	4.91	1.05 (1.00, 1.09)	0.027
Smoking					
Ex-smoker vs nonsmoker	0.1825	0.2468	0.55	0.79 (0.36, 1.75)	0.460
Current smoker vs nonsmoker	−0.6016	0.2604	5.34	0.36 (0.16, 0.84)	0.021
Exposure: MDI/TDI vs HDI	0.6062	0.1925	9.91	3.36 (1.58, 7.15)	0.002
Months of exposure	−0.0023	0.0015	2.28	1.00 (1.00, 1.00)	0.131
NAT2 rs2410556	0.1549	0.1998	0.60	1.36 (0.62, 2.98)	0.438
NAT2 rs4271002	0.4462	0.1951	5.23	2.44 (1.14, 5.24)	0.022
NAT1 rs8190845	−0.8295	0.4509	3.38	0.19 (0.03, 1.12)	0.066
NAT2 rs13277605	0.1204	0.2152	0.31	1.27 (0.55, 2.96)	0.576
NAT1 rs8190845**NAT2* rs13277605	2.1275	1.0175	4.37	8.39 (1.14, 61.7)†	0.036

CI, confidence interval; DA, diisocyanate asthma; HDI, hexamethylene diisocyanate; MDI, methylene diphenyl diisocyanate; NAT, N-acetyltransferase; TDI, toluene diisocyanate; OR, odds ratio.

*Adjusted for smoking status and type of exposure (HDI vs MDI or TDI).

†Odds of DA+ when minor alleles are present at both SNPs vs when only major alleles are present at one or both SNPs. Goodness of fit: chi-square = 8.01 with 8 *df*, $P = 0.432$.

marker for DA susceptibility.[36] Wikman et al[37] studied the role of *NAT* genotypes in the development of DA in 182 diisocyanate-exposed workers; 109 diagnosed with DA and 73 with no asthma symptoms. The authors found a significant effect of the slow acetylator *NAT1* genotype on DA (OR 2.54; 95% CI, 1.32, 4.91). This effect was especially marked in workers exposed to TDI (OR 7.77; 95% CI, 1.18, 51.6). They also assessed the effect of *NAT* genotypes in combination with the previously examined *GST* genotypes. The combination of the *GSTM1* null genotype with *NAT1* (OR 4.53, 95% CI, 1.76 to 11.6), *NAT2* (OR 3.12, 95% CI, 1.11 to 8.78) or *NAT1* and *NAT2* slow acetylator genotypes (OR 4.20, 95% CI, 1.51 to 11.6) conferred an increased risk for DA. This was the first report showing the importance of *NAT* genotypes individually or in combination with GST genotypes in DA. Two SNPs overlapping between this and our study, rs1801280 (*NAT2*∗5) and rs1041983 (*NAT*∗7), were not significantly associated with DA in our analysis.

Among our significant SNPs, only the *NAT2* rs4271002 has been previously investigated and found to be associated with risk of non-OA phenotypes.[38,39] The *NAT2* rs4271002 SNP was associated with an increased risk of asthma associated with paracetamol treatment in infancy.[38] Kim et al found that the *NAT2* rs4271002 SNP and a haplotype carrying this variant were significantly associated with aspirin exacerbated asthma (ORs 1.61 and 1.62, respectively).[39] They reported a putative binding site in DNA sequence for candidate transcription factor, upstream stimulatory factor (USF)-1. USFs are key regulatory elements of the transcriptional mechanism and presumed to play an important role in the development of bronchial asthma.[40] In our analysis, RegulomeDB gave ''no data'' score for the rs4271002 SNP. The role of this variant and its possible modifying effect on gene expression in workers with DA is unknown.

The other significant SNP, rs2410556, showed a minimal evidence for protein binding (RegulomeDB score = 6). It falls, however, within both BCL6 (B-Cell CLL/Lymphoma 6) and Tcf1 (T cell factor 1) binding motifs. BCL6 is a transcription factor that plays an important role in the regulation of follicular helper T cells and Th2 responses.[41,42,43] Tcf1 is another transcription factor that is critical for T cell development.[44] The association of this SNP with DA could be related to its overlap with these binding motifs.

The *NAT2* rs13277605 and *NAT1* rs11777998 SNPs in the combined genotypes also showed minimal evidence for protein binding in RegulomeDB (score 6). They were, however, located in the binding motifs for SPI1 (Spi-1 Proto-Oncogene) and Hoxd10 (and 13) (Homeobox D), respectively. Both transcription factors are known to be involved in cell development and differentiation.[45,46] No functional information was found for the rs8190845 SNP. Therefore, it is difficult to postulate the underlying interaction between these SNPs. The overlap of the *NAT2* rs13277605 and *NAT1* rs11777998 SNPs with these binding motifs, however, may underlie the interaction and play a role in the evolution of DA.

The major strengths of this study include a well-defined phenotype, and examination of candidate genes based on their functional role in disease pathogenesis. In addition to comparing with exposed workers without any evidence of respiratory disease, we were able to incorporate a comparator worker group (DA−) with respiratory symptoms not caused by diisocyanate exposure confirmed by negative SIC testing. We were also able to test our genetic associations while adjusting for potential independent confounding factors such as atopy, smoking history, exposure duration, and specific diisocyanate exposure. The major limitations include small sample size because of rarity of DA, and the issue of multiple interferences. Also, small numbers of subjects carrying specific alleles or genotype combinations resulted in large confidence intervals. Another limitation is that the AW controls were younger and had less exposure to diisocyanates than cases. This was unintentional because of difficulty in the recruitment of age-matched workplace controls and may be problematic in terms of detection of age-related associations. The results were not corrected for multiple comparisons because our analyses were based on well-defined roles of the selected genes in disease process. Instead, we reported all tests that reached the 0.05 level of significance.

Taken together, this case-control study reports that the *NAT2* variants and their interactions may be important in susceptibility to DA supporting the hypothesis that genetic variability influencing oxidative balance contributes to the pathogenesis of this disease. Further studies are warranted to confirm these findings in an independent replication cohort and to characterize functional role of these markers in DA and other chemically induced OA phenotypes.

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