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N-acetyltransferase 2 genotypes are associated with diisocyanate-induced asthma

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Abstract

Objective—To investigate whether genetic variants of N-acetyl transferase genes (NAT1 and NAT2) are associated with diisocyanate asthma (DA).

Methods—The study population consisted of 354 diisocyanate-exposed workers. Genotyping was performed on genomic DNA, using a 5′ nuclease PCR assay.

Results—The NAT2 rs2410556 and NAT2 rs4271002 variants were significantly associated with DA in univariate analysis. In the first logistic regression model comparing DA+ and AW groups, the genotype combination, NAT2 rs2410556 and NAT2 rs4271002, showed association with DA risk (p=0.005). In the second model comparing DA+ and DA− groups, NAT2 rs4271002 and NAT2 rs13277605 variants were significantly associated with an increased risk of DA (p=0.002 and p=0.027, respectively). In the third model comparing DA− and AW groups, the NAT1 rs4921580 SNP and the combined genotype NAT2 rs2410556/rs4271002 showed association with the DA− phenotype (p=0.017, p<0.001, respectively).

Conclusion—These findings suggest that variations in the NAT2 gene and their interactions contribute to DA susceptibility.

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CONFLICTS OF INTEREST

There are no conflicts of interests declared.

Keywords

diisocyanates; occupational asthma; NAT1; NAT2; single nucleotide polymorphism; genetics; lung; toluene diisocyanate; 4,4′- diphenylmethane diisocyanate; hexamethylene diisocyanate

INTRODUCTION

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 10–25% 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 5–15 % of them with chronic exposure develop occupational asthma[3–6].

Current evidence suggests that the pathophysiology of diisocyanate-induced 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. showed the formation of H_2O_2 in white blood cells of subjects after diisocyanate exposure[12]. Another study reported altered expression of proteins involved in oxidant/anti-oxidant-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 inter-individual 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]. Since 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 SNPs 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 comprised 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 controls (AWs). The main study analyses were conducted on only Caucasian French Canadian workers (n=354) to avoid the possibility of bias due to population stratification[24]; supplemental analyses were also conducted on the entire sample, as described below. 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 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 that had a minor allele frequency >5% and an r^2>0.8 in 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 in order to minimize bias due to population stratification. The numbers of subjects recruited from other non-Caucasian-French Canadian (n = 31) and Spanish (n = 26) populations were too small to

independently support statistical model development. However, the same logistic models were fit to the entire sample and results of those are included as supplementary data. Potential associations between each SNP and DA were tested using chi-square tests for single SNP associations. Because of the low prevalence of some of the minor alleles, a dominant model for each SNP was used for further statistical analyses. That is, each SNP was dichotomized as either 1) heterozygotes or homozygotes involving the minor allele or 2) major allele homozygotes. The sample size did not support including all possible two-way interactions between pairs of SNPs in a logistic regression model, therefore, interactions were screened using Breslow-Day tests for homogeneity of odds ratios. Logistic regression models predicting DA status were built using backward elimination, with the starting list of potential predictors including SNPs having significant single association with DA and main effects and two-way interactions of SNPs having significant heterogeneity of odds ratios, and all models also included demographic variables that were significantly associated with DA model [age, smoking status, and type of diisocyanate exposure (HDI vs. MDI or TDI) or length of exposure]. SNPs and their interactions having $p < 0.05$ were retained in the model, as were main effects of SNPs involved in significant interactions. In the first model, comparison of DA+ and AW controls was conducted whereas DA+ and DA− symptomatic groups and DA− and AW controls were compared in the second and third models, respectively. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). SNAP was used to find proxy SNPs within 500kb 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; $p < 0.001$). 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 $p=0.852$). The prevalence of smoking was significantly different between DA+ and AW controls ($p<0.001$). The overall type and the severity of the respiratory symptoms (e.g., 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.

Table 2 shows the distribution of genotypes in the study population and the p-values represent the comparison of the proportions of genotypes between two groups. The *NAT2* rs24110556 and rs4271002 SNPs were the only candidate SNPs that were individually significantly associated with the DA diagnosis. The distribution of the *NAT2* rs2410556 genotype was significantly different in DA+ workers compared to DA− group ($p=0.008$),

and AW controls (p<0.001). The distribution of the *NAT2* rs4271002 SNP was significantly different among DA+ cases compared to DA− group (p=0.001), and AW controls (P<0.001).

Tables 3–5 present logistic regression models examining statistically significant SNPs and interactions associated with DA after adjusting for significant confounders (age, smoking status, type or duration of exposure). 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-French Canadians are shown in Supplementary Tables 3–5.

The first logistic regression model included DA+ and AW groups and adjusted the results for age, smoking status and length of exposure (Table 3). Only HDI-induced DA+ cases were taken into consideration since controls were exposed only to HDI. The co-presence of minor alleles of the *NAT2* rs2410556 and rs4271002 SNPs was associated with an increased risk of DA (Odds ratio (OR), 30.62; 95% confidence interval (CI), 2.84- 330).

The second model included DA+ and DA− groups and adjusted the results for smoking status and type of diisocyanate exposure (HDI vs. MDI or TDI) (Table 4). The carriage of the minor alleles for the *NAT2* rs4271002 and *NAT2* rs13277605 SNPs was associated with an increased risk of DA with ORs of 2.77 (95% CI, 1.45–5.30, p=0.002) and 2.21 (95% CI, 1.09–4.46, p=0.027), respectively.

The third model included DA− and AW groups and adjusted the results for age at diagnosis and length of exposure (Table 5). Only HDI-induced DA− subjects were taken into consideration since controls were exposed only to HDI. The *NAT1* rs4921580 SNP was associated with a decreased risk of DA− phenotype with an OR of 0.30 (95% CI, 0.11–0.81, p=0.017). In addition, the carriage of the minor alleles for the *NAT2* rs4271002 and *NAT2* rs2410556 SNPs was associated with susceptibility to DA− phenotype with an OR of 38.0 (95% CI, 5.6–258, p<0.001).

The four 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 r2 of 1). This led to the identification of an additional 7 correlated SNPs using data from the International HapMap Project. The total set of 11 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]. The rs4921580 and one proxy SNP (rs62492997) had a RegulomeDB score of 3a (based on the following available datatypes; TF binding + any motif + DNase peak). 6 SNPs (rs13277605, rs2410556, rs78344578, rs4345600, rs79533018, rs11780272) 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, rs4546703) 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 the univariate analysis when evaluated against two control comparator groups (i.e., AW and DA− groups). A multivariate analysis was then applied adjusting for age, smoking status and length of exposure. Here, differences emerged between comparator groups in *NAT2* genotypes associated with DA. With the AW group as a comparator, only the combined genotype *NAT2* rs2410556/rs4271002 was significantly associated with DA. The *NAT2* rs4271002 variant, however, remained significantly associated with DA when compared with the DA− group.

While the *NAT2* rs2410556 SNP was individually associated with DA in the univariate analysis, this effect was seen only in combination with the rs4271002 SNP in the multivariate analysis. Increased risk related to this variant combination is context-dependent and suggests that some SNPs display significant association when considered as part of a SNP-covariate or SNP-SNP interaction. The *NAT2* rs13277605 and *NAT1* rs4921580 SNPs were also significant in the second and third logistic regression models. These results suggest previously unrecognized associations of NAT genotypes with the symptomatic workers population. The functional consequence and the role of these SNPs in asthmatic 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]. A recent meta-analysis showed that slow acetylator NAT2 genotypes might increase asthma risk among Caucasians (OR 2.20; 95 % CI 1.31–3.72)[34]. To our knowledge, there have been two other studies examining the role of *NAT* variants in DA. Earlier, Berode et al. reported that *NAT2* slow acetylator individuals, exposed to common diisocyanate monomers at work, are more susceptible to asthma[35]. Later, they confirmed their finding in a larger study and showed that *NAT2* slow acetylation could be a surrogate marker for DA susceptibility[36]. Wikman et al. 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[37]. The authors found a significant effect of the slow acetylator *NAT1* genotype on DA (OR: 2.54; 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: 1.76–11.6), NAT2 (OR: 3.12, 95% CI: 1.11- 8.78) or *NAT1* and *NAT2* slow acetylator genotypes (OR: 4.20, 95% CI: 1.51–11.6) 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-occupational asthma 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, Regulome DB gave 'no data' score for the rs4271002 SNP. However, SNAP search showed that there is a strong LD (r^2=1) between the rs4271002 and *NAT2* rs62492997 SNPs. RegulomeDB cites that rs62492997 SNP affects binding of ESR1 (estrogen receptor 1) protein and alters the Nr2f2 binding motif. Nr2f2, a ligand inducible transcription factor that is involved in the regulation of many different genes, plays critical roles in cell differentiation and is known to be differentially expressed in asthma[41]. ESR1 polymorphisms were found to be associated with airway hyperresponsiveness and lung function decline[42]. This SNP was individually associated with DA in the univariate analysis. In addition, it conferred increased risk for DA individually (with an OR of 2.77) in the second logistic regression model and in combination with rs2410556 SNP in the first and third logistic regression models. It is noteworthy that the frequency of the combined genotype *NAT2* rs2410556/rs4271002 was significantly greater in workers with DA− vs. AWs, a finding likely explained by a high incidence of non-occupational asthma among DA− subjects.

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 due to 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 due to 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 since 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 occupational asthma phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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TABLE 1

Characteristics of study participants

	DA+	DA−	AWs	DA+ vs DA− p-values	DA+ vs AWs p-values	Overall p-values
N	95	117	142			
Sex, M/F	84/11	106/11	132/10	0.605	0.228	0.484
Age at Diagnosis ± s.e.	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, months ± s.e.	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-years ± s.e.	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			

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TABLE 2

Distribution of genotype frequencies between the groups (French-Canadians only)

Gene/SNP ID	DA+ (n = 95)	DA− (n = 117)	AWs (n=142)	χ^2 or Fisher's exact p-values	
	N (%)	N (%)	N (%)	DA+ vs. DA−	DA+ vs. AWs
NAT1 rs11777998				0.701	0.769
GG	70 (94.5)	93 (80.2)	106 (75.6)		
GC	23 (24.5)	22 (19.0)	32 (22.5)		
CC	1 (1.1)	1 (0.9)	4 (2.8)		
NAT1 rs13253389				0.845	0.299
GG	42 (44.2)	52 (45.2)	52 (36.6)		
GA	45 (47.4)	51 (44.4)	70 (49.3)		
AA	8 (8.4)	12 (10.4)	20 (14.1)		
NAT1 rs4298522				0.197	0.468
TT	46 (48.4)	42 (36.2)	62 (44.0)		
TA	41 (43.2)	63 (54.3)	60 (42.6)		
AA	8 (8.4)	11 (9.5)	19 (13.5)		
NAT1 rs4921580				0.957	0.437
CC	75 (78.9)	90 (78.3)	104 (73.2)		
CG	17 (17.9)	22 (19.1)	35 (24.6)		
GG	3 (3.2)	3 (2.6)	3 (2.1)		
NAT1 rs4921880				0.252	0.838
AA	53 (55.8)	76 (65.5)	83 (58.4)		
AT	35 (36.8)	36 (31.0)	51 (35.9)		
TT	7 (7.4)	4 (3.5)	8 (5.6)		
NAT1 rs7003890				0.854	0.426
TT	31 (32.6)	34 (29.1)	47 (33.1)		
TC	48 (50.5)	62 (53.0)	62 (43.7)		
CC	16 (16.8)	21 (17.9)	33 (23.2)		
NAT1 rs7017402				0.734	0.788
GG	73 (76.8)	90 (77.6)	105 (73.9)		
AG	21 (22.1)	23 (19.8)	34 (23.9)		

Gene/SNP ID	DA+ (n = 95)	DA− (n = 117)	AWs (n=142)	χ^2 or Fisher's exact p-values	
	N (%)	N (%)	N (%)	DA+ vs. DA−	DA+ vs. AWs
AA	1 (1.1)	3 (2.6)	3 (2.1)		
NAT1 rs8190837				0.129	0.528
AA	80 (84.2)	106 (91.4)	126 (88.7)		
AG	13 (13.7)	10 (8.6)	15 (10.6)		
GG	2 (2.1)	0 (0.0)	1 (0.7)		
NAT1 rs8190845				0.793	0.910
GG	73 (76.8)	92 (78.6)	111 (78.7)		
AG	20 (21.1)	24 (20.5)	27 (19.2)		
AA	2 (2.1)	1 (0.8)	3 (2.1)		
NAT1 rs9325827				0.954	0.735
TT	68 (72.3)	82 (70.7)	99 (69.7)		
CT	24 (25.5)	32 (27.6)	37 (26.1)		
CC	2 (2.1)	2 (1.7)	6 (4.2)		
NAT2 rs13277605				0.190	0.505
GG	21 (22.6)	38 (33.6)	41 (29.3)		
GT	48 (51.6)	53 (46.9)	68 (48.6)		
TT	24 (25.8)	22 (19.5)	31 (22.1)		
NAT2 rs1801280				0.305	0.678
TT	26 (27.4)	25 (21.6)	34 (23.9)		
TC	48 (50.5)	55 (47.4)	70 (49.3)		
CC	21 (22.1)	36 (31.0)	38 (26.8)		
NAT2 rs1961456				0.617	0.636
AA	53 (56.4)	57 (49.6)	88 (62.0)		
GA	34 (36.2)	48 (41.7)	43 (30.3)		
GG	7 (7.5)	10 (8.7)	11 (7.7)		
NAT2 rs2410556				**0.008**	**<0.001**
TT	24 (25.5)	51 (45.1)	100 (70.9)		
CT	31 (33.0)	33 (29.2)	35 (24.8)		
CC	39 (41.5)	29 (25.7)	6 (4.3)		
NAT2 rs4271002				**0.001**	**<0.001**

Gene/SNP ID	DA+ (n = 95)	DA− (n = 117)	AWs (n=142)	χ^2 or Fisher's exact p-values	
	N (%)	N (%)	N (%)	DA+ vs. DA−	DA+ vs. AWs
GG	27 (28.7)	58 (50.9)	110 (77.5)		
GC	14 (14.9)	19 (16.7)	29 (20.4)		
CC	53 (56.4)	37 (32.5)	3 (2.1)		
NAT2 rs4646246				0.572	0.385
AA	69 (72.6)	77 (66.4)	113 (80.1)		
AG	23 (24.2)	35 (30.2)	24 (17.0)		
GG	3 (3.2)	4 (3.4)	4 (2.8)		
NAT2 rs1799930				0.276	0.289
GG	55 (58.5)	58 (49.6)	77 (54.2)		
GA	34 (36.2)	47 (40.2)	49 (34.5)		
AA	5 (5.3)	12 (10.3)	16 (11.3)		
NAT2 rs1799931				0.615	0.686
GG	92 (96.8)	109 (94.0)	139 (97.9)		
GA	3 (3.2)	6 (5.2)	3 (2.1)		
AA	0 (0.0)	1 (0.9)	0 (0.0)		

TABLE 3

Logistic regression model for significant variations in HDI exposed workers: DA+ (n=49) vs AW controls (n=138)

Model term	Estimate, β	S.E.	χ^2	OR (95% CI)	p-value
Intercept	−5.3689	1.1718	20.99		**<0.001**
NAT2 rs4271002	0.0940	0.3924	0.06	1.21 (0.26, 5.62)	0.811
NAT2 rs2410556	0.0629	0.3759	0.03	1.13 (0.26, 4.95)	0.867
NAT2 rs4271002 * NAT2 rs2410556	3.4216	1.2127	7.96	30.62 (2.84, 330)	**0.005**

†Adjusted for age, smoking status and length of exposure

TABLE 4

Logistic regression model for significant variations, DA+ (n=90) vs DA− (n=108) groups

Model term	Estimate, β	S.E.	χ^2	OR (95% CI)	p-value
Intercept	−0.3154	0.1942	2.64		0.104
NAT2 rs4271002	0.5102	0.1650	9.56	2.77 (1.45, 5.30)	**0.002**
NAT2 rs13277605	0.3961	0.1794	4.88	2.21 (1.09, 4.46)	**0.027**

†Adjusted for smoking status and type of exposure (HDI versus MDI or TDI)

TABLE 5

Logistic regression model for significant variations in HDI exposed workers: DA− (n=86) vs AW controls (n=138)

Model term	Estimate, β	S.E.	χ^2	OR (95% CI)	p-value
Intercept	−5.8264	0.9403	38.4		<0.001
NAT2 rs4271002	−0.0968	0.3425	0.1	0.82 (0.22, 3.16)	0.778
NAT2 rs2410556	0.0703	0.2583	0.1	1.15 (0.42, 3.17)	0.786
NAT1 rs4921580	−0.5976	0.2503	5.7	0.30 (0.11, 0.81)	**0.017**
NAT2 rs4271002* NAT2 rs2410556	3.6384	0.9777	13.8	38.0 (5.6, 258)	**<0.001**

†Adjusted for age at diagnosis and length of exposure