**Interferon-γ Promoter Is Hypermethylated in Blood DNA from Workers with Confirmed Diisocyanate Asthma**

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Risk factors have not been identified that determine susceptibility for development of diisocyanate-induced occupational asthma (DA). We hypothesized that diisocyanate (DI) exposure could modify gene promoter regions regulating transcription of cytokine mediators and thereby influence expression of DA. A cross-sectional study was designed to investigate the promoter methylation status of candidate genes in DI-exposed workers. Subjects consisted of 131 workers in three groups: 40 cases with DA confirmed by a positive specific inhalation challenge (SIC) (DA+), 41 exposed workers with lower respiratory symptoms and negative SIC (DA−), and 50 asymptomatic exposed workers (AWs). We studied four candidate genes (GSTM1, DUSP22, IFN-γ, and IL-4) for which altered promoter methylation has been previously investigated for relationships with a variety of other environmental exposures. Methylation status was determined using methylation-specific quantitative PCR performed on genomic DNA extracted from whole blood. Results showed that relative methylation of IFN-γ promoter was significantly increased in DA+ in comparison with both comparator groups (DA− and AW), and it exhibited good sensitivity (77.5%) and specificity (80%) for identifying DA workers in a multivariate predictive model after adjusting for type of DI exposure, smoking status, methacholine PC_{20} and gender. IL-4 promoter was slightly less methylated only in DA+ compared with AW among nonsmoking workers. Both GSTM1 and DUSP22 promoter methylations were found not associated with DA. Our finding suggests that exposure to occupational chemicals could play a heretofore undefined mechanistic role via epigenetic modification of specific genes in the promoter region.

Key Words: DNA methylation; IFN-γ; environmental exposure; diisocyanate; asthma; occupational asthma.

**Disclaimer:** The findings and conclusions in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veteran’s Affairs or the U.S. government.

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studies of promoter regions of multiple candidate genes including glutathione S-transferase Mu 1 (GSTM1), dual specificity protein phosphatase 22 (DUSP22), and interferon-γ (IFN-γ) conducted in professional firefighters chronically exposed to smoke-related toxicants (Ouyang et al., 2012). We found significantly reduced DUSP22 promoter methylation relative to nonfirefighters. In a birth cohort study, maternal exposure to traffic-related polycyclic aromatic hydrocarbons was found to be associated with promoter hypermethylation in both the acyl-CoA synthetase long-chain family member 3 (ACSL3) and the IFN-γ gene in umbilical cord blood DNA from offspring, although no such effect was observed in the interleukin-4 (IL-4) gene (Perera et al., 2009; Tang et al., 2012). Finally, exposure of young children to traffic-related, diesel exhaust particles was found associated with forhead box P3 (FOXP3) promoter hypermethylation in saliva DNA of children that developed wheezing and asthma (Brunst et al., 2013).

We posited that specific epigenetic changes induced by DI in genomic DNA of exposed workers could be associated with DA. To test this hypothesis, we investigated methylation status using four candidate gene promoters (GSTM1, DUSP22, IFN-γ, and IL-4), which have been studied in firefighters or traffic-related offspring asthma (Ouyang et al., 2012; Perera et al., 2009; Tang et al., 2012) and assessed their associations with DA, through a cross-sectional study of DI-exposed workers. The choice of these genes was based on prior observations that the methylation status of their promoters is susceptible to alteration upon exposure to complex mixtures of environmental agents.

**MATERIALS AND METHODS**

**Subjects.** Subjects are the workers with occupational exposure to one of the common DIIs, MDI, TDI, or HDI in the workplace. They were recruited from occupational pulmonary disease clinics located in Canada (Sacre Coeur Hospital, Montreal, and Laval Hospital, Sainte-Foy, Quebec). To confirm or exclude DA, all subjects underwent specific inhalation challenge (SIC) with the appropriate work-relevant DI chemicals according to previously described protocols (Malo et al., 1999; Sastre et al., 2003). Three groups of subjects were studied, consisting of: 40 workers with DA confirmed by a positive SIC test and were categorized in the DA+ group, 41 workers presenting with work-related lower respiratory symptoms and negative SICs and were categorized in the DA− group, and 50 asymptomatic HDI-exposed spray painters were recruited as AWs. Ten workers in each group were current smokers, whereas the remainder had never smoked. Demographic characteristics of the three study groups are listed in Table 1. All participants gave signed informed consent using protocols approved by the ethics committee at each respective institution.

Five-ten milliliters of EDTA-anticoagulated blood was collected from each subject and stored frozen at −80°C prior to DNA extraction. Skin prick testing was performed in participants with a panel of common aeroallergens; atopy was defined as a positive skin test (wheal ≥ 3 mm) to at least one aeroallergen. Airway hyperresponsiveness was evaluated by methacholine inhalation challenge testing performed prior to SIC testing and expressed as the provocative concentration of methacholine eliciting a fall in forced expiratory volume in 1 s (FEV1) of ≥ 20% from baseline challenge baseline (PC_{20}).

**DNA methylation.** DNA was purified using Flexigene DNA kits from Qiagen, according to the manufacturer's instructions. Methylation status of gene promoters was analyzed using methylation-specific quantitative PCR (MS-qPCR) (Ng et al., 2011). For DNA bisulfite conversion, 500 ng genomic DNA from each sample was bisulfite converted and eluted in 50 μl of elution solution using the EZ Methylation Kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions. Primers for MS-qPCR of GSTM1, DUSP22, and IFN-γ were described in previous report (Ouyang et al., 2012) and primers for MS-qPCR of IL-4 were designed based on sequence from GenBank NC_000005.9: forward methylated: 5'-GATGGAGATTATTTGTTGTAATACG; reverse methylated: 5'-GCCTCCTAAATCTACGCCT; forward unmethylated: 5'-GATGGAGATTATTTGTTGTAATATG; reverse unmethylated: 5'-TCCACTCTTAAATCTACCT (NG-qPCR was carried out in a total volume of 10 μl, containing 5 μl 2× Power SYBR Green Master Mix (Invitrogen), 0.5 μl 10 μM “methylated-specific” or “unmethylated-specific” primers, 0.2 μl ROX, 2.3 μl H₂O, and 2 μl of bisulfite-converted DNA template in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) cycled at 50°C × 2 min, 95°C × 10 min and followed by 45 cycles of 95°C × 15 s and 60°C × 1 min. Data were analyzed using Sequence Detector v2.3 Analysis Software (Applied Biosystems). Each sample was analyzed in duplicate. DNA from the prostate cell line, LNCaP; was used in each set reaction as the internal control. Relative level of methylation of a gene promoter in each sample was calculated using the equation: % methylation = 100 × (2^ΔCt(meth-unmeth))/1 × 100% and was used as the primary measure in this article (Ng et al., 2011). Hypermethylation was defined as ≥ 50% methylated.

**Data analysis.** Relative methylation levels of four candidate genes were considered primary measures of interest and they are all numerical variables. Each of the primary measures was assessed of its association to the DA status, a categorical variable of three groups (DA+, DA−, and AW) using a univariate fixed effect (or one-way ANOVA) model. Post hoc means were compared between groups under the fixed effect model framework. Considering more than two groups were compared simultaneously, a correction method called Tukey’s method was used to account for multiple comparison and ensure an overall type I error of 0.05. In order to identify confounders or adjusting covariates for multivariate analyses, the following methods were used on characteristic variables listed in Table 1: (1) each of the characteristic variable was assessed of its association to the DA status using a chi-square test if it was a categorical variable or an ANOVA model if it was a numerical variable and (2) each of the primary measures was assessed of its association to a categorized characteristic variable using an ANOVA model. A characteristic variable showing significance in any of proposed analyses was considered an adjusting covariate. The associations between primary measures and the DA status were then reassessed using multivariate fixed effect models after including the adjusting covariates as independent variables. In addition, using each adjusting covariate as strata, both univariate and multivariate fixed effect models were repeated in subgroups stratified by this variable. For example, as smoking status (smoking vs. nonsmoking) was identified as an adjusting covariate, subanalyses were performed among smokers and on smokers individually. Since in the end, both GSTM1 and DUSP22 showed no significant associations to the DA status in any analysis using either univariate models or multivariate models and either the overall method including all subjects or subset methods including a proportion of subjects only; their results were not reported in the article. IL-4 showed limited association to the DA status, specifically among the nonsmokers only, hence its results were only reported in Supplemental Table 1. IFN-γ was used to predict DA (a binary variable of DA+ vs. DA−) using univariate and multivariate logistical regression models, respectively. A propensity score (or the predicted probability) of DA+ was generated from each of the logistical regression models and used to construct the receiver operating characteristic (ROC) curve to evaluate the performance of identifying or predicting DA+ using the methylation of IFN-γ promoter. The accuracy of identification was assessed using the area of the ROC curve (area under the curve [AUC]), and the sensitivity and specificity corresponding to a cut of the propensity score. All statistical analyses were performed using SAS 9.3 (SAS, Cary, NC). p Values < 0.05 were considered statistically significant.
RESULTS

Subjects and Characteristics

Group descriptive characteristics are shown in Table 1. One hundred and thirty-one Caucasian subjects with median age of 31 (range 19–64) years and a male:female ratio of 115:16 were studied. Subjects were categorized into three test groups: 40 with SIC confirmed DA (DA+), 41 symptomatic workers in whom DA was excluded (DA−) based on a negative SIC test with a work-relevant DI chemical, and 50 asymptomatic exposed workers (AWs). Because we anticipated that cigarette smoking may impact the DNA methylation status of candidate genes, 10 currently smoking workers in each group were recruited. Workers were classified by specific chemical exposure at work (i.e., MDI, TDI, and HDI). All AWs were exposed to HDI at work, whereas 47% of the DA+ subjects were exposed to HDI, 25% to MDI, and 27.5% to TDI. In the DA− group, 68.3% were exposed to HDI, 17.1% were exposed to MDI, and 14.6% to TDI. The overall duration of DI exposure was significantly higher in the AW group (p = 0.044); however, there was no difference in duration of exposure to HDI among the three study groups (p = 0.893). The DA+ group had significantly greater number of subjects with hyperresponsiveness to methacholine defined by a PC_{20} (mg/ml) levels < 2 mg/ml than other groups (p < 0.001).

DNA Methylation Studies

Relative promoter methylation levels of four candidate genes (GSTM1, DUSP22, IFN-γ, and IL-4) were assessed in 131 DI-exposed workers. The GSTM1 promoter was sparsely methylated in all samples (mean ± SE = 8.1 ± 2.9%), whereas the DUSP22 promoter region was heavily methylated in all samples (mean ± SE = 83.2 ± 2.5%). Both showed no statistical associations to the DA status (DUSP22: F-statistic = 0.86, p_F = 0.43; GSTM1: F-statistic = 0.38, p_F = 0.69). The IL-4 methylation was not significantly associated to the DA status in overall analyses when all workers were included (see Supplementary table 1 in the Online Supplementary Data). However, in a subset including nonsmokers only, the DA+ group showed slightly lower methylation level (mean ± SE = 5.0 ± 1.8%) than the AW group (mean ± SE = 12.1 ± 1.5%, p's < 0.05 using univariate and multivariate models, respectively). Interestingly, higher methylation level was found in the DA+ group in a univariate model but not in multivariate model when only smokers were studied.

Associations of the IFN-γ methylation to demographic and clinical characteristics of interest were presented in Table 2. There were higher relative methylation levels among smokers and workers with lower mean baseline methacholine PC_{20}.

In addition, the relative methylation of IFN-γ promoter was also found significantly associated with type of DI exposure and gender. Four variables, smoking status, methacholine PC_{20}, type of DI exposure, and gender, were used as adjusting covariates in the multivariate models for evaluating associations between methylation of IFN-γ promoter and DA status. Table 3 summarized mean ± SE of IFN-γ methylation among three DA groups and their comparisons using univariate and multivariate models, respectively. In particular, the mean ± SE of IFN-γ methylation was 59.2 ± 4.1% in the DA+, higher than 38.1 ± 4.0% and 40.1 ± 3.6% in the DA− and AW groups, respectively, using the univariate model. Similar conclusions could be found from the multivariate model except that the difference between DA+ and AWs was not significant (p = 0.126). Subset analyses showed the IFN-γ methylation was significantly associated to the DA status in subgroups such as nonsmokers, or workers with absence of airway hyperresponsiveness (defined as PC_{20} 32 mg/ml), or male participants, or workers with MDI exposure only.
In the predictive analyses, we evaluated the performance of the IFN-γ promoter methylation as a predictor of DA+ phenotype using AUC from the ROC analysis. Figure 1 and Table 4 showed the moderate accuracy of identifying DA+ using the IFN-γ promoter methylation (univariate model), with an AUC of 0.71. In a multivariate logistical regression model, DA+ phenotype was predicted by IFN-γ promoter methylation, along with other covariates (or predictors) of gender, smoking status, exposure, and methacholine PC_{20}. Contributions of the covariates were assessed using the Wald statistics and they were 7.87 ($p = 0.005$), 2.1 ($p = 0.147$), 2.5 ($p = 0.114$), 15.2 ($p = 0.001$), and 18.9 ($p < 0.001$) for the predictors of IFN-γ promoter methylation, gender, smoking status, exposure, and methacholine PC_{20}, respectively. The AUC from this multivariate model increased (0.71–0.87) as did sensitivity (60–77.5%). Interestingly, IFN-γ promoter methylation seemed to have the best prediction of DA for the workers with methacholine PC_{20} of ≥ 32, with AUCs reaching 0.81 and 0.94 in the univariate and multivariate models, respectively.

**DISCUSSION**

OA is defined as “variable airflow limitation and/or airway hyperresponsiveness due to exposure to a specific causal agent present in a particular work environment and not to stimuli encountered outside the workplace” (Tarlo et al., 2008). IgE-mediated OA due to a workplace respiratory sensitizer is more often attributable to protein sensitizers rather than reactive chemicals. Allergic asthma is a disorder of airway inflammation and airway hyperresponsiveness driven by Th2 cytokines and specific IgE induced by exposure and sensitization to allergens. Enhanced expression of IL-4 has been identified in bronchial biopsies of patients with mild asthma but not moderate or severe asthma (Siddiqui et al., 2010). DI chemicals including HDI, TDI, and MDI are prototypic reactive chemicals known to cause DA. Despite clinical features suggesting an allergic mechanism, serum-specific IgE antibodies for DI-HSA antigens have been demonstrated in less than 30% of workers with confirmed DA (Bernstein et al., 2002). In addition, although IL-5 is enhanced, IL-4 and IgE expression is not increased in bronchial biopsy tissue of workers with DA following SIC with TDI (Jones et al., 2006). The latter observation suggests that

### Table 2

**IFN-γ Methylation Level (%) to Demographic and Clinical Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Category</th>
<th>IFN-γ Mean ± SE</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>&lt; 30</td>
<td>42.9 ± 3.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>≥ 30</td>
<td>47.4 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>58.0 ± 6.7</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>43.6 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>DI exposed</td>
<td>HDI</td>
<td>41.7 ± 2.7</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>MDI</td>
<td>61.1 ± 6.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TDI</td>
<td>50.3 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td>Current</td>
<td>57.5 ± 4.7</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>41.5 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Atopy</td>
<td>Negative</td>
<td>47.5 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>44.0 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>Methacholine PC_{20} (mg/ml)</td>
<td>&lt; 2</td>
<td>52.6 ± 5.3</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>≥ 2</td>
<td>49.9 ± 3.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 32</td>
<td>38.6 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Duration of DI exposure</td>
<td>&lt; 72</td>
<td>44.9 ± 3.5</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>≥ 72</td>
<td>45.7 ± 3.3</td>
<td></td>
</tr>
</tbody>
</table>

*Note. NS, no statistical significant with a $p$ value > 0.05.*

### Table 3

**IFN-γ Methylation Level (%) Among DA+, DA−, and AWs**

<table>
<thead>
<tr>
<th>Stratification variable</th>
<th>Category</th>
<th>(1) DA+ Mean ± SE</th>
<th>(2) DA− Mean ± SE</th>
<th>(3) AW Mean ± SE</th>
<th>$p$ value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>—</td>
<td>59.2 ± 4.1</td>
<td>38.1 ± 4.0</td>
<td>40.1 ± 3.6</td>
<td>0.001/0.007</td>
</tr>
<tr>
<td>DI exposed</td>
<td>HDI</td>
<td>53.4 ± 5.9</td>
<td>36.5 ± 4.9</td>
<td>40.1 ± 3.7</td>
<td>0.077/0.221</td>
</tr>
<tr>
<td></td>
<td>MDI</td>
<td>73.1 ± 6.0</td>
<td>43.9 ± 7.1</td>
<td>—</td>
<td>0.007/0.007</td>
</tr>
<tr>
<td></td>
<td>TDI</td>
<td>56.6 ± 9.1</td>
<td>38.8 ± 12.4</td>
<td>—</td>
<td>0.265/0.097</td>
</tr>
<tr>
<td>Smoking status</td>
<td>Never</td>
<td>58.6 ± 4.4</td>
<td>31.3 ± 4.4</td>
<td>36.5 ± 3.8</td>
<td>0.000/0.002</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>61.0 ± 8.4</td>
<td>56.9 ± 8.0</td>
<td>54.8 ± 8.4</td>
<td>0.935/0.987</td>
</tr>
<tr>
<td>Methacholine PC_{20} (mg/ml)</td>
<td>≥ 32</td>
<td>64.3 ± 8.5</td>
<td>33.7 ± 5.3</td>
<td>35.7 ± 4.1</td>
<td>0.010/0.034</td>
</tr>
<tr>
<td></td>
<td>&lt; 2</td>
<td>58.5 ± 7.9</td>
<td>43.9 ± 13.3</td>
<td>29.2 ± 23.0</td>
<td>0.226/0.191</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>68.6 ± 9.8</td>
<td>55.1 ± 10.8</td>
<td>48.4 ± 10.8</td>
<td>0.636/0.439</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>57.6 ± 4.4</td>
<td>35.8 ± 4.3</td>
<td>39.2 ± 3.8</td>
<td>0.002/0.026</td>
</tr>
</tbody>
</table>

*Note. $p$ Values were obtained from both univariate/multivariate fixed effect model. In the univariate model, the group (DA+ vs. DA− vs. AW) factor was the only independent variable of interest, whereas in the multivariate method, other factors such as DI exposed, smoking, methacholine PC_{20} (mg/ml), and gender were used as controlling covariate or independent variables. Both $p$ values were accounted for multiple comparisons using a Tukey’s method to ensure an overall 5% type I error.

<sup>a</sup>Univariate/multivariate.
alternate non-IgE, non-IL-4-dependent immune mechanisms may contribute to airway inflammation in DA. We and others have reported that DIs enhance release of proinflammatory cytokines and chemokines (e.g., monocyte chemoattractant protein-1) from monocytes and macrophages. Nonimmune direct oxidative stress effects of DIs on respiratory epithelium may also play an important role in the pathogenesis of DA (Bernstein et al., 2002; Lumnus et al., 2011; Wisnewski et al., 2005, 2008).

In this study, we found significantly higher levels of IFN-γ promoter methylation in DA+ workers than in DA− and AWs. In contrast, methylation of IL-4 promoter was slightly decreased in nonsmoking workers with DA+, indicating that epigenetic modification of IL-4 was not significant in these workers. The actual biologic role of IFN-γ, however, in development and perpetuation of DA is unknown. This data would suggest that decreased production of IFN-γ via hypermethylation and silencing of its gene promoter could enhance Th2 differentiation and Th2 cytokine-directed airway inflammation. Although we did not investigate the entire epigenome in this study, it is probable that epigenetic modifications of multiple genes could also be associated with DA.

Overall, IFN-γ promoter percent methylation was generally higher in smoking workers than nonsmoking workers (see Table 2). When levels of methylation of IFN-γ promoter were compared among DA groups, it was interesting to find they were only significant among workers who never smoked, whereas for smokers, the levels of methylation of IFN-γ promoter were similar among the various groups (Table 3). These data suggested that environmental tobacco smoke exposure had a substantial effect on IFN-γ promoter methylation, which is similar to the report that secondhand smoke in combination with ambient air pollution exposure is associated with increased CpG methylation and decreased expression of IFN-γ in T effector cells and Foxp3 in T regulatory cells in children (Kohli et al., 2012). However, for nonsmoking subjects, the DA (or group) effect was noticeably a significant factor contributing to IFN-γ promoter methylation.

Currently, there are no validated diagnostic tests that accurately identify workers with DA. Our data showed that IFN-γ promoter methylation could be a potential marker for DA, and further studies are needed to confirm these findings. Overall, our results suggest that nonimmune direct oxidative stress effects of DIs, along with cytokine and chemokine release from monocytes and macrophages, may play an important role in the pathogenesis of DA.
promoter methylation could be promising marker to enhance the accuracy in distinguishing DA+ from DA-exposed workers. Obviously, smoking status and female gender are also associated with hypermethylation of IFN-γ promoter, which could confound the ability of this assay to differentiate DA+ and non-DA workers. However, using a multivariate model to adjust for other covariate predictors (Table 3) sufficiently improved test characteristics to achieve 77.5% sensitivity and 80% specificity. Our initial data also suggest that the IFN-γ hypermethylation test should be further evaluated in expanded worker cohorts as a potential biomarker of risk for DA.

There are several limitations intrinsic to the design of this type of a cross-sectional study. Hypermethylation of IFN-γ (defined as ≥ 50% methylated) was by no means specific for DA and was identified in 17% of symptomatic exposed workers (DA+) as well as AWs (23%) in our comparator groups. These findings suggest that other environmental toxicants, encountered in both occupational and nonoccupational settings, might also contribute to IFN-γ promoter methylation. This finding may not be specific for OA. Runyon et al. (2012) reported that T cells collected from adult discordant asthmatic twins demonstrated increased methylation of the IFN-γ. We have recently reported that IFN-γ promoter hypermethylation of leukocytes obtained from human neonates participating in a birth cohort study was associated with maternal exposure to polycyclic aromatic hydrocarbons. Another limitation is the unknown effect of different frequencies of exposure to MDI and TDI versus HDI between the DA+ group and the two comparator groups (see Table 1). Finally, because this is a cross-sectional study, we were unable to assess dose-response effects of DI exposure on IFN-γ promoter methylation.

The role of hypermethylation of the IFN-γ promoter in the pathogenesis or clinical expression of DA remains to be determined in future studies. It has been demonstrated that in vivo and in vitro exposure to DIs, specifically MDI, readily form adducts with nuclear DNA. Thus, although unproven, it is feasible that DIs or aromatic diamine derivatives reacting with DNA could directly bind to CpG sites in the IFN-γ gene promoter region (Bolognesi et al., 2001). The finding of hypermethylation in the majority of DA+ patients is consistent with a hypothesis that exposure to occupational chemicals could play a heretofore undefined role in enhancing airway inflammation potentially via epigenetic modification of the IFN-γ promoter region. If the relatively high sensitivity and specificity demonstrated in this study is replicated in future prospective longitudinal studies, IFN-γ hypermethylation could become a biomarker for predicting risk of DA.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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REFERENCES


diisocyanate-induced occupational asthma. Int. Arch. Allergy Immunol. 150, 156–163.


