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Prospective Study of Metal Fume-Induced Responses of Global Gene Expression Profiling in Whole Blood

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Abstract

Metal particulate inhalation causes pulmonary and cardiovascular diseases. Our previous results showed that systemic responses to short-term occupational welding-fume exposure could be assessed by microarray analyses in whole-blood total RNA sampled before and after exposure. To expand our understanding of the duration of particulate-induced gene expression changes, we conducted a study using a similar population 1 yr after the original study and extended our observations in the postexposure period. We recruited 15 individuals with welding fume exposure and 7 nonexposed individuals. Thirteen of the 22 individuals (9 in exposed group and 4 in nonexposed group) had been monitored in the previous study. Whole-blood total RNA was analyzed at 3 time points, including baseline, immediately following exposure (approximately 5 h after baseline), and 24 h after baseline, using cDNA microarray technology. We replicated the patterns of Gene Ontology (GO) terms associated with response to stimulus, cell death, phosphorus metabolism, localization, and regulation of biological processes significantly enriched with altered genes in the nonsmoking exposed group. Most of the identified genes had opposite expression changes between the exposure and postexposure periods in nonsmoking welders. In addition, we found dose-dependent patterns that were affected by smoking status. In conclusion, short-term occupational exposure to metal particulates causes systemic responses in the peripheral blood. Furthermore, the acute particulate-induced effects on gene expression profiling were transient in nonsmoking welders, with most effects diminishing within 19 h following exposure.

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Ambient particulate exposure increases morbidity and mortality from respiratory and cardiovascular diseases (Samet et al., 2000; Peters et al., 2001a). The welding process generates high levels of respirable metal particulate, and is associated with increased prevalence of inflammatory lung diseases and adverse cardiovascular effects (El-Zein et al., 2003; Antonini, 2003; Donaldson et al., 2005). A cohort study demonstrated that occupational exposure to welding fumes significantly increased the risk of myocardial infarction (Sjogren et al., 2002). It has been proposed that inhaled particulates from air pollution may cause systemic alterations, in addition to effects on the autonomic nervous system, by releasing inflammatory cytokines subsequent to pulmonary inflammation (Magari et al., 2001; Liao et al., 2005). Systemic inflammation, in turn, plays an important role in the pathogenesis of atherosclerosis and coronary diseases. Currently, very little is known about the human genetic network and pathways underlying such systemic inflammation in humans exposed to either ambient fine particles or metal particulates. In a previous study, we conducted an exploratory analysis using cDNA microarray techniques to analyze global gene expression profiling on whole blood total RNA in a group of healthy individuals before and after occupational exposure to welding fume (Wang et al., 2005). We found that acute occupational exposure to metal particulates caused alterations in the expression of several genes clustered in functional pathways related to pro-inflammatory and immune responses, oxidative stress, phosphate metabolism, cell proliferation, and programmed cell death. These results support the hypothesis that there are systemic reactions occurring in the peripheral blood in response to metal particulate exposure.

Our previous, exploratory study was the first to demonstrate that detectable changes in gene expression profiling could be measured in human whole-blood total RNA using a paired sampling study design (collecting both pre- and postexposure samples from the same individual) even though the particulate-induced changes are small in comparison to person-to-person variations. However, it was not clear whether the particulate-induced expression changes represent solely short-term responses or whether there are any longer term effects. The current study is an extension of the previous study. In addition to collecting samples at the baseline and immediately postexposure as in the previous study, samples were also collected at a third time point 24 h after the baseline, about 19 h after cessation of exposure. The three sampling times allowed us to monitor the time course of the gene expression changes by comparing profiles during the exposure period (immediately postexposure versus baseline), the postexposure period (24 h after baseline versus immediately postexposure), and the entire study period (24 h after baseline versus baseline). The results of the extended observations enable us to more completely assess the temporal pattern of systemic responses as determined by the whole-blood RNA global expression profiling in response to metal particulate exposure.

One of the major challenges of our microarray research is the issue of high dimensionality of the data due to the extremely large number of variables measured in a relatively small number of samples. There is also a high degree of interrelations among tested genes (Li & Luan, 2005; Mello-Coelho & Hess, 2005). In our previous study, after initial filtering with paired *t*-tests, we applied an alternative strategy of examining the genes passing this filter in the context of underlying biological processes based on prior knowledge in the database of the Gene Ontology (GO) consortium (Ashburner et al., 2000). Our study demonstrates that GO biological process analysis is a powerful approach for determining responses to metal particulate exposure. Several recent publications have also applied GO terms in the analyses of gene expression data (Anderson et al., 2004; Koskinen et al., 2004; Krasnov et al., 2005; Sadlier et al., 2004). A similar approach named Gene Set Enrichment Analysis (GSEA) was developed at the Broad Institute of Massachusetts Institute of Technology and Harvard using the Molecular Signature Database (MSigDb), which contains megolic and signaling pathways from 10 public databases (Subramanian et al., 2005). GSEA was applied to the analysis of microarray data from diabetic muscle biopsies, and the results have been independently

validated by other microarray studies and in vivo functional studies (Mootha et al., 2003; Patti et al., 2003; Petersen et al., 2004). Although the functional annotation of genes is still an ongoing process, this extended study nevertheless enables us to gain more insight into the functional pathways involved in occupational metal exposure.

METHODS

Study Population

Study participants were recruited from welding workshops held on Saturdays at an apprentice welding school as described previously (Wang et al., 2005; Kim et al., 2005). Welders were subjects who were exposed to metal fume and airborne fine particulate matter mainly from shielded metal arc (stick), gas metal arc welding (TIG), and plasma arc or acetylene torch cutting and grinding, using base metals of carbon steel and stainless steel (manganese, chromium, and nickel alloys) with electrodes composed mainly of iron and variable amounts of manganese (1–5%). The study participants included 15 welders and 7 nonexposed subjects who stayed in the office or classroom of the same building during the measured period. Among them, 9 exposed individuals and 4 nonexposed individuals had also participated in the previous study. Before the day of sample collection, all nonexposed controls and 14 out of 15 welders had at least a 7-day washout window without any welding or boilermaking work. Only one smoking welder performed approximately 5 h stick and TIG welding 1 day before the sampling day. Blood samples were collected from each subject before (baseline) and immediately after the welding workshop (~5 h from baseline) from all subjects. Only 13 of 22 subjects (8 nonsmoking welders, 3 smoking welders, and 2 nonexposed controls) contributed blood samples 24 h after baseline, as there was no welding workshop the following day. There were no significant differences in age ($p = .29$), smoking status ($p = .66$), and years as boilermaker ($p = .07$) between these 13 subjects with 24 h after baseline samples and the remaining 9 subjects who did not return for sampling at 24 h after baseline. Three time periods were defined as follows: the exposure period (~5 h between baseline and immediately postexposure), the postexposure period (~19 h between immediately postexposure and 24 h after baseline), and the entire study period (between baseline and 24 h after baseline). A modified American Thoracic Society (ATS) questionnaire was used to obtain relevant medical information, current cardiopulmonary symptoms reported by study subjects, smoking history, and occupational history (Ferris, 1978). Procedures for complete blood counts on all blood samples were described previously (Kim et al., 2005). Approval of the study protocol was obtained from the Institutional Review Board of the Harvard School of Public Health, and written informed consent was obtained from each subject.

Exposure Assessment

During the welding session, welders did not wear any kind of respirator, except a personal face protection shield. Exposure to particulate matter (with a mass median aerodynamic diameter $\leq 2.5\mu$m, PM2.5) was monitored for each subject using Dust-Trak Aerosol Monitor (TSI, Inc., St. Paul, MN) fitted with a 2.5-μm pore size filter in line with a Vortex Timer 2 personal sampling pump (Casella USA, Amherst, NH) calibrated at a flow rate of 3.5 L/min, as described previously (Kim et al., 2004). The inlet tubing of the personal monitor was secured to the participant's shoulder in the breathing zone area. Before the study, the DustTrak monitors were calibrated by the manufacturer and we conducted daily zero balance and flow checks. The mass of air sample collected on the filter, weighed before and after sampling, was divided by the air volume sampled to calculate the gravimetric PM2.5 concentration.

Microarray Hybridization and Data Analysis

Protocols for sample collection and processing, RNA extraction, and quality assessment were described previously (Wang et al., 2005). We used Affymetrix Human Genome U133A

GeneChips (Affymetrix, Santa Clara, CA) in genome-wide expression profiling, which was carried out at the Microarray Core Facility of the Dana-Farber Cancer Institute, Boston. All RNA samples collected from each subject were processed together in one batch of microarray analysis to minimize inherent variations. After an initial quality check, we used the DNA-Chip Analyzer 2006 (dChip, http://www.dchip.org) software, which applied an invariant set of genes for normalization and calculation of expression values across all microarrays, to normalize raw microarray signals with the assumption that a subset of genes had constant expression among all subtype of cell (Li & Wong, 2001). The Detection Calls of a gene (Present Calls or Absent Calls) in an RNA sample were carried out by Affymetrix MAS 5.0 software using the one-sided Wilcoxon signed-ranked algorithm (Liu et al., 2002).

All data analyses were conducted on a subset of 4,663 probe sets (of a total of 22,215 probe sets on Affymetrix U133A microarray), with Present Calls in over 50% of tested arrays. Differentially expressed genes between 2 time points were identified by initially screening for average fold changes of paired samples larger than 1.25 or less than −1.25, and then tested genes using a one-sided paired *t*-test with a *p* value <.05 as the cutoff for significance. This approach proved to be a more reliable ranking criterion for gene selection in the MicroArray Quality Control (MAQC) project, and was used as a filter to identify the differentially expressed genes carried forward for the clustering analyses using GO terms (Guo et al., 2006; Shi et al., 2006).

Clustering Analyses Using Gene Ontology

We used a modified functional clustering analyses based on the annotations defined by the GO Consortium (http://www.geneontology.org) (Ashburner et al., 2000). Briefly, the probability of observing a particular number of genes in a single GO biological process from the paired *t*-test results was tested using the hypergeometric distribution as previously described (Tavazoie et al., 1999). To ensure valid tests, we tried to avoid GO terms with either very large or very small number of annotated genes by focusing only on GO terms with less than 1000 annotated genes on the Affymetrix U133A GeneChips and at least 5 annotated genes in the testing gene lists. A significant GO biological process was identified after strict Bonferroni correction. GeneNotes software (http://combio.cs.brandeis.edu/GeneNotes/index.htm) was used in the analyses (Hong & Wong, 2005).

Statistical Analysis

Statistical analyses were performed using SAS version 9 (SAS Institute, Inc., Cary, NC). Exposure status was dichotomized as nonexposed controls and exposed welders. Study population characteristics and $PM_{2.5}$ exposure between controls and welders, as well as smokers and nonsmokers, were compared using two-sample *t*-tests, Wilcoxon rank-sum tests with exact *p* values, and Fisher's exact test. The mean (with standard deviation, SD) values of the $PM_{2.5}$ concentrations were determined. The level of significance for all analyses was set at .05.

RESULTS

An expanded study of response to welding fume was conducted 1 yr after the initial exploratory study in this population (Wang et al., 2005). The population demographic data are summarized in Table 1. With comparable median sampling times between nonexposed controls and welders (4.4 vs. 4.6 h, respectively, $p = .642$), the median $PM_{2.5}$ concentrations were significantly higher among welders ($p < .0001$) than among nonexposed controls. There were no significant differences in $PM_{2.5}$ concentrations by smoking status in the exposed group ($p = .988$). In addition to welding, the welders also spent a large amount of time doing grinding work, which largely generates particles in the coarse fraction rather than the fine fraction; therefore, their

exposure to $PM_{2.5}$ was significantly lower (median $PM_{2.5}$: 0.948 mg/m^3; range: 0.331–2.894 mg/m^3) than in the previous study in which welding was primarily performed (median $PM_{2.5}$: 2.44 mg/m^3; range: 1.301–3.42 mg/m^3) ($p < .0001$).

Initially, we screened differentially expressed genes during the exposure period, which was approximately 5 h between baseline and immediately postexposure (the exposure period), by fold change coupled with one-side paired *t*-tests in both the exposed (welding) and nonexposed groups. Since our previous study found that exposure to welding fume was significantly associated with the increased cell counts of total white blood cells and neutrophils in nonsmoking welders, but not in smoking welders, who had significantly higher baseline cell counts, all analyses were stratified by smoking status (Wang et al., 2005). Compared with nonexposed controls, both nonsmoking and smoking welders had increased numbers of differentially expressed genes (Table 2), which were similar to the results of the previous study. Furthermore, there were similar levels of metal fume induced gene expression changes in nonsmoking welders as measured by the ratios of altered genes in the welders to the nonexposed controls between this study (4.7-fold) and the previous study (4.1-fold). However, we found a lower level of metal fume induced gene expression changes in smoking welders in this study (1.6-fold) than the previous study (5.6-fold).

We then assessed global expression variations by investigating the distribution of paired *p* values (two-sided) of all genes (4,663 probe sets) over the exposure period. We expected to see a uniform distribution from 0 to 1 under the null hypothesis of no difference among the nonsmoking nonexposed controls. Instead, we were surprised to observe that this distribution was skewed toward large *p* values, and a similar distribution was also observed in the nonexposed controls of the previous study (Figure 1). In contrast, we found a shift in the distribution toward small *p* values in nonsmoking welders for both studies; slightly more genes shifted toward small *p* values in the previous study, corresponding to higher $PM_{2.5}$ exposure. Furthermore, the shift in *p*-value distribution was also observed in smoking welders when compared with the nonsmoking nonexposed controls. Higher levels of exposure in the previous study caused observable shifts in the *p*-value distribution in smoking welders but to a lesser degree compared with nonsmoking welders. However, with a lower level of exposure in this study, the *p*-value distribution among smoking welders is almost indistinguishable from nonsmoking nonexposed controls (Figure 1). This finding suggested that smoking altered expression profiles in whole blood total RNA and a higher level welding exposure was required to reach the same level of global shift of the *p*-value distribution observed in nonsmoking welders.

To further examine the 24-h time point, we focused on the nonsmoking welders to identify any genes with altered expressions in the postexposure period and across the entire study period (~24 h). When compared with the results of the exposure period of nonsmoking welders (154 genes from 159 probe sets), there were more genes (201 genes from 213 probe sets) with altered expression during the postexposure period, Relatively few genes changed during the entire study period (40 genes from 40 probe sets), which is comparable to the observations seen in the nonexposed controls. Eighty-two genes showed significant opposite directional changes in gene expression in both the exposure period and the postexposure period. Four upregulated genes were found in both the exposure period and the entire study period and 10 downregulated genes were common in the postexposure period and the entire study period. Moreover, from the expression variation plot of the exposure period versus the postexposure period (Figure 2), we found that most of the altered genes identified from each period, 98% of the exposure period and 99% of the postexposure period, showed the trend of opposite directional change in expression in the other period as measured by average fold changes even though the changes were not statistically significant in paired *t*-tests ($p < .05$). Furthermore, we observed a similar degree of shift in the *p*-value distribution toward small *p* values during the postexposure period

(Figure 3). In contrast, there was a relatively flat *p*-value distribution during the entire study period. Taken together, these observations suggest that acute metal fume exposure induces transient changes of gene expression profiling in whole blood total RNA in nonsmoking welders, with the baseline expression profiling being almost fully restored 19-h after the exposure.

Gene lists generated by screening were analyzed by hypergeometric distribution testing to define any GO biological processes significantly enriched with the filtered genes. In general, we found that GO biological processes that were tested significantly in more than one gene list tended to have close interconnections on GO structure under a general GO term, whereas those found from only one gene list tended to be more specific GO terms and sporadically distributed in the lower part of GO structure. GO biological processes that were tested significantly in more than one gene list were summarized in Table 3. In this study, we only observed significant enriched GO biological processes in nonsmoking welders, with most of GO terms clustered in five groups, including: Response to Stimulus (GO: 50896), Death (GO: 16265), Phosphorus Metabolism (GO: 6793), Localization (GO: 51179), and Regulation of Biological Process (GO: 50789). There were similar patterns of significant GO terms identified during the exposure period and postexposure period, except in the group Localization (GO: 51179), which was specific only to the exposure period. With fewer altered genes identified during the entire study period (between baseline and 24 h after baseline), significant GO biological processes only appeared in the groups of Death (GO: 16265) and Localization (GO: 51179).

In the previous study, significant GO biological processes were found in four of five GO groups in nonsmoking welders, except the group Death (GO: 16265). In addition, the group RNA metabolism (GO: 16070) and Catabolism (GO: 9056) was observed in the previous study but not in the current study (data not show). In smoking welders, significant GO terms were observed in all six GO groups in the previous study, but not in the current study. Considering the small number of genes obtained from nonexposed controls in both studies and in smoking welders of this study, which might result in less significant GO terms identified, we raised the paired *p*-value cutoff from .05 to .1 to obtain larger gene lists. With increased number of genes, there were still only a small number of sporadic GO terms tested significantly in nonexposed controls of both studies and smoking welders of this study. This is contrary to a large number of significant GO terms in nonsmoking welders. In contrast, more significant GO terms were found in smoking welders in the previous study with similar patterns of nonsmoking welders (Table 3). The difference in significant GO terms in smoking welders between the previous study and the current study correlated well with the significantly higher level of metal fume exposure seen in the previous study.

DISCUSSION

In this study, we expanded our previous study using the same field protocols for sample collection within a similar population. Using fold changes coupled with one-sided paired *t*-test as initial filtering methods, there were consistent findings in both studies that more genes could be detected in the whole-blood total RNA of nonsmoking welders than in the nonexposed controls. Increased alteration of the genes identified in both nonsmoking and smoking welders in the previous study correlated with significantly higher levels of exposure. This is consistent with the observed changes in global expression profiling by *p*-value distribution of all genes (Figure 1). Moreover, there were similar clustering patterns of the filtered genes in the groups of GO biological processes. Thus, our current study confirms our previous findings that short-term occupational exposure to metal particulates causes global gene expression profile changes in peripheral blood.

The major finding of the current study was that the acute particulate-induced effects on blood gene expression profiling lasted for a short period in nonsmoking welders, with most of the effects diminishing 19 h after cessation of the exposure. Although we could not directly compare the results with nonexposed controls due to the small number of samples collected at 24 h after baseline, the data from nonsmoking welders collected at 3 time points support this conclusion. First, we observed a similar level of significant expression alteration during the 19-h postexposure period (201 genes) and the 5-h exposure period (154 genes), but fewer changes during the entire 24-h study period (40 genes) (Figure 3). Second, all of the common significant genes in both the exposure period and the postexposure period had expression changes in opposite directions (Figure 2). Finally, particulate-induced expression alterations during the exposure period and the postexposure period were clustered in the same groups of GO biological processes (Table 3). In addition, there were similar transient changes of systemic inflammatory markers measured in the same occupational cohort, which observed significant increases in white blood cell (WBC) and neutrophil counts and a significant decrease in fibrinogen level in nonsmoking welders during the exposure period but no significant difference during the entire 24-h study period (Kim, 2005). Our results were also consistent with previous epidemiological findings that increased levels of systemic inflammatory markers were associated with elevated ambient particulate levels (Pekkanen et al., 2000;Peters et al., 2001;Schwartz, 2001;Seaton et al., 1999).

In nonsmoking welders, the number of filtered genes was similar and the same functional pathways were significantly enriched with the identified genes in the current study and the previous study. However, we noticed that there were only a few common genes ($n = 17$) identified in both studies. The significantly lower levels ($p < .0001$) of metal particulate exposure in this study (median $PM_{2.5}$: 0.948 mg/m^3; range: 0.331–2.894 mg/m^3) than in the previous study (median $PM_{2.5}$: 2.44 mg/m^3; range: 1.301–3.42 mg/m^3) or exposure to slightly different compositions of metal particulates between these studies might be the causes for this discordance. Unfortunately, the sample sizes of both studies were not large enough to conduct further gene-responsive analyses based on the exposure dosages or stratified analyses by different types of metal particulates. Since all of the subjects in this study and the previous study were recruited from the apprentice workshop at the same welding school, we assumed there were similar particle compositions in the exposed welding fume of these studies. Although it was possible that batch effects between studies might contribute to the observation of less common genes, we believed it was not a significant confounding factor for the following reasons: the same sampling protocols were applied in both studies; all RNA samples collected from each subject were processed together in one batch of microarray analysis; the small numbers of altered genes were found in nonexposed controls in both studies; and the global expression profiling variations correlated well with the levels of exposure and smoking status.

On the other hand, a lack of concordant genes might imply that there were no major gene effects in response to acute welding fume exposure. Instead, in response to a particular environmental challenge, the expression of a group of functionally relevant genes might have changed since genes are highly networked and coordinated in nature. The cDNA microarray technique, which simultaneously measures thousands of gene expressions, is only a snapshot of dynamically changing global gene expression profiles at the time of RNA sample stabilization. The frequently observed larger interpersonal variations in microarray analyses actually represent different global gene expression profiles at the time of RNA sample stabilization, which make it very difficult for the analysis in a traditional case-control study design where subtle differences are expected between cases and controls. Thus, a repeated-sampling strategy and self-pairing study design should be considered for population-based studies generating data with high dimensionality and high degree of interrelations. In our metal particulate exposure studies, each baseline microarray data represented a unique expression profile at the beginning of exposure, with individual genes expressed possibly at different levels among study subjects.

Since that acute metal particulate exposure caused small and transient changes in the whole-blood total RNA, the expression alterations in networked genes of the relevant functional groups were expected. Some genes changed uniformly among study subjects, resulting in small paired *p* values, whereas some did not change uniformly, with large paired *p* values. Several observations supported directly or indirectly this hypothesis. First, there were global shifts of the paired *p*-value distribution toward small paired *p*-values in the exposed welders, which was sensitive to the level of exposure and smoking status. Second, there were similar patterns of GO biological processes enriched with the altered genes in both studies. Finally, most genes that had significant alternations in the exposure (or postexposure) period but not in the postexposure (or exposure) period tended to change in the opposite direction.

The skewed distribution of the *p* values from the paired *t*-tests between immediately postexposure and baseline from nonexposed controls in both studies was very interesting since it was quite different from the expected null distribution. The distributions skewed toward large *p* values not only indicated there is no additional factor influencing the blood gene expression between two time points in these studies, but it also suggested there were strong interrelationships among genes. Indeed, we always found strong correlations among genes regardless of their paired *p*values, exposure status, and smoking status (data not shown). The observations of strong correlations of genes suggested we could not test individual gene expression in a highly correlated setting, whereas, by testing for the enrichment of genes in certain pathways, we could effectively accommodate the existence of multiple correlated genes. In addition, the levels of the global shift in the *p*-value distribution were related to the exposure level and smoking status, suggesting that the exposure-induced changes in gene expression were not limited to those genes identified as the most significant by fold changes coupled with paired *t*-tests. The pathway-based approach was recently adopted in microarray analyses to elucidate the functional pathways enriched with genes having small expression changes in a given experimental system, since a single gene would be difficult to detect accurately under such circumstances (Moggs, 2005; Li et al., 2004). Determining the association between certain GO terms and differentially expressed genes from microarray assays would be an ideal way to understand the molecular and functional processes implied by the gene expression changes (Anderson et al., 2004). Comparing the current study with our previous study, the observation of a high degree of agreement in the GO terms enriched with metal-particulate responsive genes, other than individual altered genes, clearly demonstrated the usefulness of the pathway-based approach in microarray analyses. However, as GO terms are subjected to continuous update, more robust methods for GO pathway analysis need to be developed and evaluated.

In contrast to the nonsmoking welders, smoking welders showed fewer welding fume induced changes in gene expression, as revealed by the number of filtered genes and global shifts of the paired *p*-value distribution. The different responses in gene expression between the nonsmoking and smoking welder were consistent with the different responses in systemic inflammatory markers measured in the same occupational cohort, with significant increases in total WBC and neutrophil counts and a significant decrease in fibrinogen level only observed in nonsmoking welders but not in smoking welders during the exposure period (Kim, 2005). In the same study, smokers had a significantly higher baseline WBC count, which was consistent with the findings from a large epidemiological study (Frohlich et al., 2003). Exposure to smoking seems to not only increase baseline WBC counts, but it might also reset baseline gene expression. Thus, a higher level of welding fume exposure is required to reach the same level of expression changes observed in nonsmokers.

Compared to the nonexposed controls, welders had more intensive physical activity during the comparable exposure period (~5 h between baseline and immediately postexposure). Extra physical activity might possibly contribute to the observed changes in the gene expression, as

previous study found that exercise could increase in the numbers of circulating lymphocytes and lymphocyte subsets, as well as enhanced gene expression in neutrophil (Sureda et al., 2007; Gleeson & Bishop, 2005). Although we collected data on physical activities for all of the subjects, the small sample size of both studies limited our capacity to conduct further gene-responsive analyses with adjustment of physical activities.

In conclusion, in response to acute welding fume exposure containing high concentrations of metal particulate, there were transient global variations in blood gene expression profiles far from the initial exposure site, which were mainly enriched with genes involved biological processes such as immune and inflammatory responses. However, the overall expression alterations were relatively small (Figure 2), together with the lack of consistency in the most significant genes that reached screening criteria between this study and the previous study. The exposure-associated pattern changes in *p*-value distribution suggested there were many more genes that had undergone exposure-induced alternations but had not reached a significant threshold (Figures 1 and 3). These observations suggest that the effects of particulate exposure on blood gene expression are both subtle and broad, and last for a short period. Precise characterization of these types of responses is a new challenge in microarray analysis of gene expression profiles in blood. Furthermore, future studies are warranted to address the health effects of this type of expression responses.

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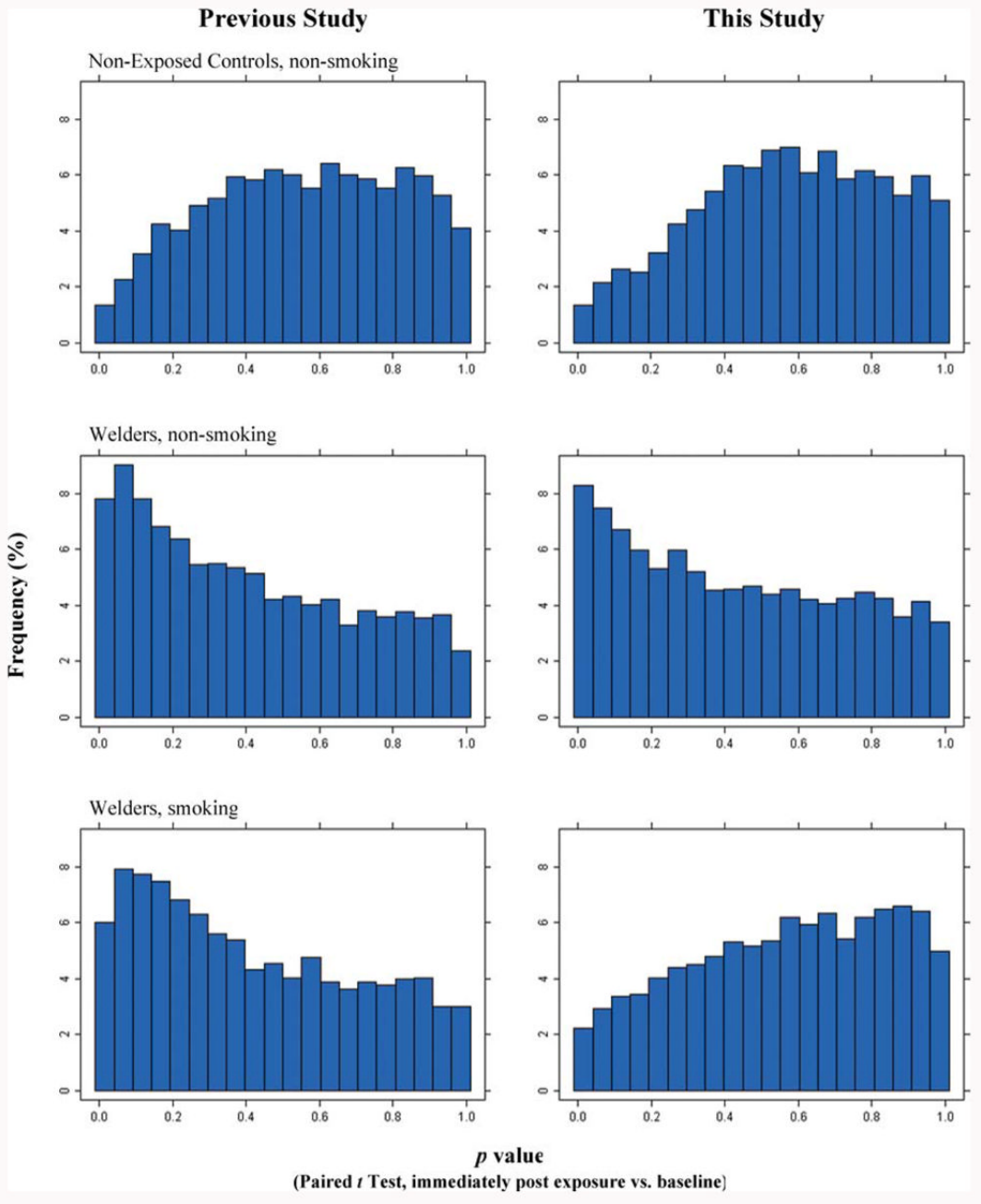

FIG. 1.

Global gene expression variations induced by welding fume exposure in this study and the previous study. Histograms of two-sided paired *t*-tests of 4663 probe sets between immediate postexposure and baseline.

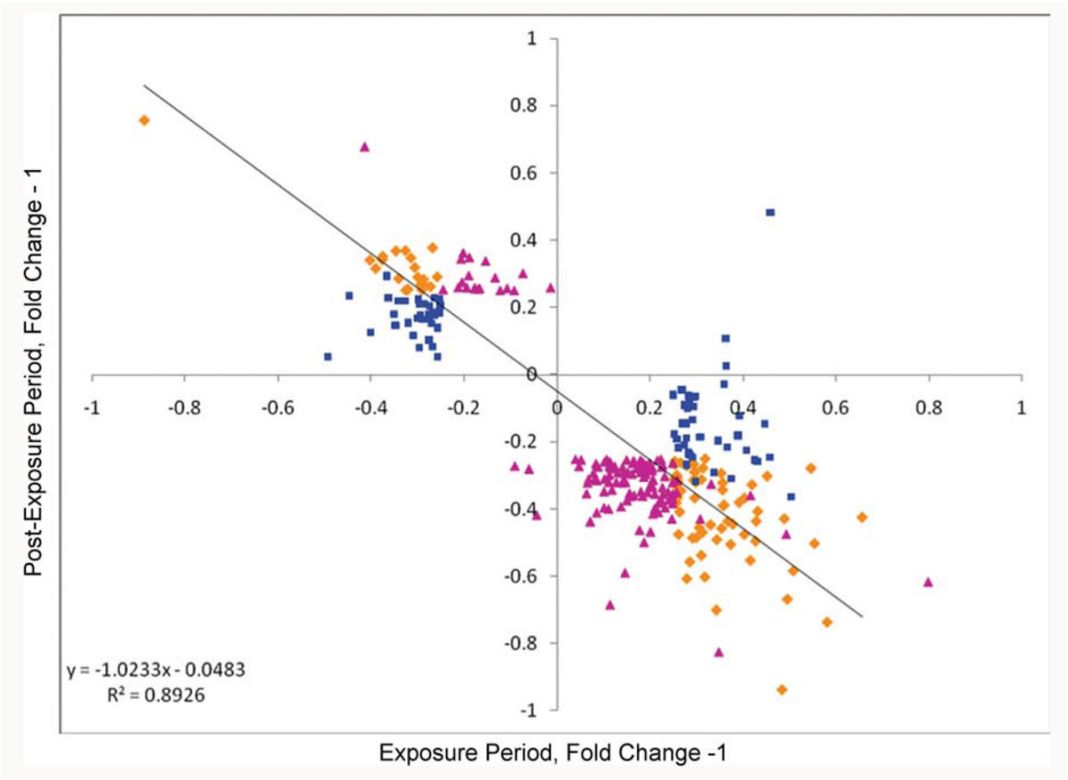

FIG. 2.

The distribution of gene expression variations during the exposure period and postexposure period. The *x*-axis is the average fold changes – 1 of gene expression at the immediately postexposure over mean expression at baseline; the *y*-axis is the average fold changes – 1 of gene expression at 24 h after baseline over mean expression at the immediately postexposure. Orange diamonds represent genes tested significantly in both the exposure and postexposure periods; blue squares represent genes tested significantly only in the exposure period; pink triangles represent genes tested significantly only in the postexposure period. The majority of genes are distributed in the upper left and lower right sections, indicating that the expression changes of those genes in the postexposure period are opposite to the changes in the exposure period.

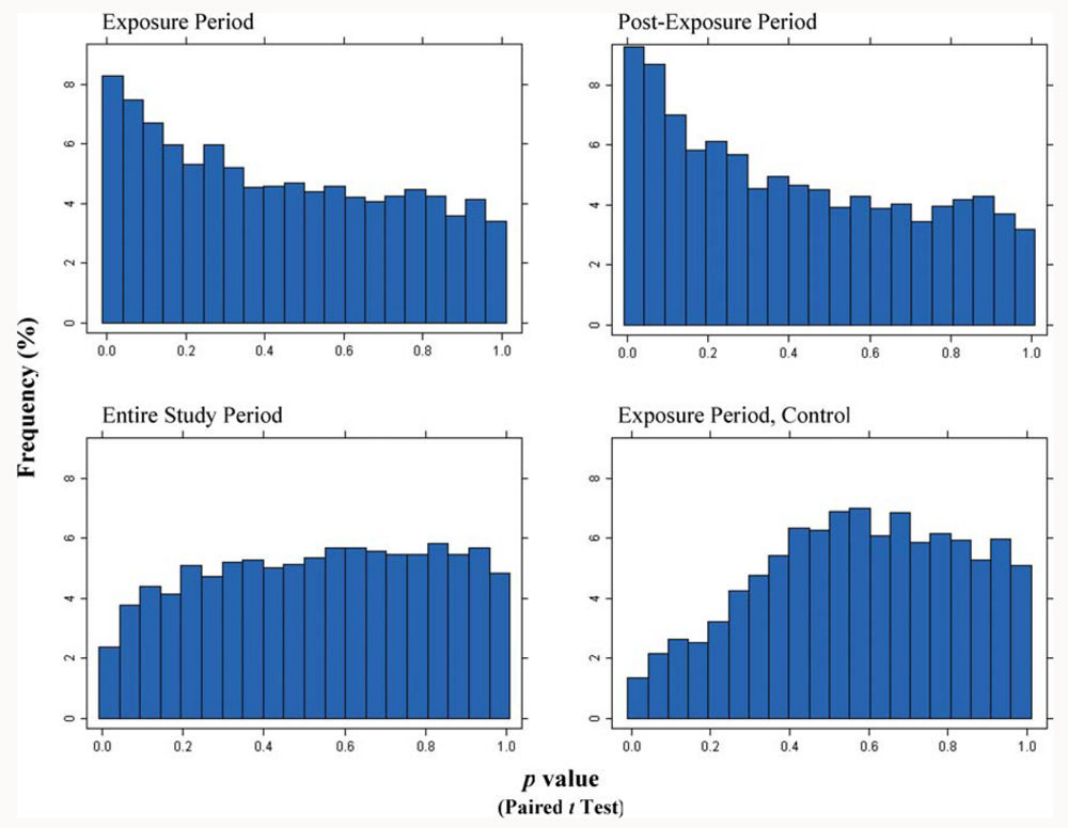

FIG. 3.

Global gene expression variations associated with welding fume exposure during different study period. Histograms of two-sided paired *t*-tests of 4,663 probe sets between different time points: (A and D) the exposure period (~5 h between baseline and immediately postexposure); (B) the postexposure period (~19 h between immediately postexposure and 24 h after baseline); and (C) the entire study period (between baseline and 24 h after baseline).

TABLE 1

Demographics of study population

Parameter	Welding group	Nonexposed group	*P*
Subjects	15	7	
Number of smokers (%)	6 (40%)	0 (0%)	.12[a]
Age, yr	39 (27–50)	47 (26–62)	.079[b]
Metal-particulate exposure (PM_{25} concentration, mg/m^3)	0.948 (0.331–2.894)	0.021 (0–0.108)	<.0001[b]
Mean (SD)	1.121 (0.693)	0.037 (0.041)	
Major chronic health problems			
Emphysema	0 (0%)	1 (14.3%)	.318[a]
Asbestosis	1 (6.7%)	1 (14.3%)	1.00[a]
Asthma	3 (10%)	0 (0%)	.52[a]
Heart attack	1 (6.7%)	1 (14.3%)	1.00[a]
Hypertension	3 (10%)	2 (28.6%)	1.00[a]
Sampling time, h			
Mean (SD)	4.6 (0.8)	4.4 (1.3)	.642[c]

Note. Unless specified, the values presented are median (range).

[a] Fisher's exact test.

[b] Wilcoxon rank-sum tests with exact *p* values.

[c] Two sample *t*-test.

TABLE 2

Genes identified by fold change coupled with paired *t*-test: Postexposure versus baseline microarrays

Group	Subjects	Genes[a] ($p < .05$)	Probe sets ($p < .05$)
This study			
Nonexposed controls	7	33	35
Nonsmoking welders	9	154	159
Smoking welders	6	52	53
Previous study			
Nonexposed controls	7	59	59
Nonsmoking welders	9	242	232
Smoking welders	6	332	356

Note. Baseline microarrays are genes with at least 50% Present Calls (dChip) in each group of paired *t*-tests with elimination of redundant probe sets.

[a]Number of genes after removing redundant probe sets with $p < .05$.

TABLE 3

Clustering analysis of genes filtered by paired *t*-test in GO biological processes*

			Nonsmoking welders				Smoking welders		Nonexposed controls	
Study (Pre: previous study; Current: this study)			**Pre**		**Curret**		**Pre**		**Pre**	**Current**
Period (Exp: exposure; Post: postexposure; Entire: entire study period)			**Exp**	**Exp**	**Post**	**Entire**	**Exp**	**Current Exp**	**Exp**	**Exp**
Nonredundant gene list			**230**	**152**	**196**	**40**	**331**	**51**	**58**	**31**
GO ID	GO term	Genes on array								
50896	**response to stimulus**	**2235**	33	23	36	9	51	5	6	4
9607	response to biotic stimulus	961	20	16	23	3	31	3	2	1
6952	defense response	917	19	14	22	3	30	3	2	1
6955	immune response	806	17	13	22	3	27	3	2	1
6954	inflammatory response	207	6	5	9	1	8	2	2	1
45087	innate immune response	66	2	1	5	0	1	0	0	0
51707	response to other organism	565	13	12	16	1	17	2	2	1
9613	response to pest, pathogen or parasite	528	12	12	16	1	17	2	2	1
9628	response to abiotic stimulus	408	10	10	6	2	16	1	0	1
42221	response to chemical stimulus	348	8	9	4	2	15	1	0	0
9605	response to external stimulus	488	10	11	12	2	16	3	2	1
9611	response to wounding	376	8	11	12	1	12	3	2	1
16265	**death**	**556**	8	13	14	5	18	4	2	2
8219	cell death	553	8	13	14	5	18	4	2	2
12501	programmed cell death	526	8	13	14	5	18	4	2	2
6915	apoptosis	524	8	13	14	5	18	4	2	2
42981	regulation of apoptosis	343	6	8	11	4	14	2	2	0
43067	regulation of programmed cell death	345	6	8	11	4	14	2	2	0
6793	**phosphorus metabolism**	**787**	15	11	15	1	19	3	6	2
6796	phosphate metabolism	787	15	11	15	1	19	3	6	2
16310	phosphorylation	640	12	11	13	1	16	3	5	1
6468	protein amino acid phosphorylation	539	9	9	11	1	13	1	5	1
51179	**localization**	**2579**	38	29	40	9	54	12	9	4
51641	cellular localization	571	13	6	7	1	19	3	2	0

			Nonsmoking welders				Smoking welders		Nonexposed controls	
Study (Pre: previous study; Current: this study)			**Pre**		**Curret**		**Pre**		**Pre**	**Current**
Period (Exp: exposure; Post: postexposure; Entire: entire study period)			**Exp**	**Exp**	**Post**	**Entire**	**Exp**	**Current Exp**	**Exp**	**Exp**
Nonredundant gene list			**230**	**152**	**196**	**40**	**331**	**51**	**58**	**31**
51649	establishment of cellular localization	565	13	6	7	1	19	3	2	0
46907	intracellular transport	558	12	6	7	1	19	3	1	0
8104	protein localization	559	12	6	12	4	19	3	3	0
45184	establishment of protein localization	543	11	6	11	4	19	3	3	0
15031	protein transport	522	11	6	11	3	19	3	3	0
51674	localization of cell	230	3	5	7	3	7	1	1	0
6928	cell motility	230	3	5	7	3	7	1	1	0
50789	**regulation of biological process**	**162**	54	35	47	7	90	11	15	6
48519	negative regulation of biological process	756	19	8	11	3	21	4	5	0
48523	negative regulation of cellular process	700	19	7	11	3	20	4	5	0
51243	negative regulation of cellular physiological process	634	17	7	10	3	20	4	5	0
43118	negative regulation of physiological process	660	17	8	10	3	20	4	5	0
48518	positive regulation of biological process	611	15	11	12	4	23	3	2	1
48522	positive regulation of cellular process	524	14	9	10	3	20	3	1	1
51242	positive regulation of cellular physiological process	442	10	9	10	3	16	3	1	0
43119	positive regulation of physiological process	459	11	9	10	3	17	3	1	0
43085	positive regulation of enzyme activity	107	1	5	5	2	3	0	1	0
51347	positive regulation of transferase activity	60	1	4	3	0	0	0	0	0
45860	positive regulation of protein kinase activity	58	1	4	3	0	0	0	0	0

Note. Orange cells indicate the GO biological processes were significantly enriched (Bonferroni correction) in the test gene lists after multiple comparison adjustment.