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Toxicogenomic Approach to Risk Assessment of Welding Fumes

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Abstract

The welding fume exposure of welders mainly occurs though the inhalation of a mixture of metals, chemicals, and dust in the workplace. Several studies have already attempted to identify the pathophysiological process of welding-fume-exposure-induced lung diseases, as well as manganism. Moreover, microarray technology offers the ability to investigate the entire genome after exposure to welding fumes, and permits characterization of the biological effects due to welding fume exposure. This review summarizes the published literature on the genetic and transcriptional profiles resulting from the exposure of cells or organisms to welding fumes as complex environmental mixtures in the workplace. Researchers are also actively studying how toxicogenomics can be effectively used for risk assessment of welding fumes, as discussed throughout this review.

Keywords: Gene expression profile; Transcription; Toxicogenomics; Welding fume; Welder

Introduction

Exposure to welding fumes is already known to produce respiratory effects, such as pulmonary function, pneumoconiosis, and lung cancer, along with certain non-respiratory results, such as manganism [1]. However, despite a large number of publications on the pathophysiological process of welding fume exposure-induced diseases to understand the disease process *in vivo* and *in vitro*, relatively few attempts have been made to determine the gene expression levels affected by welding fume exposure to identify early effect biomarkers or the differential gene expression resulting from mixed exposure.

Analyzing gene expression data on the pathway and functional levels, along with a biological systems approach, will provide a more comprehensive insight into the biological effects of exposure to complex mixtures, such as welding fumes that consist of metals, gases, dust, chemicals, and lead into an improved risk assessment of mixture exposure.

Exposure to particulate silica causes persistent inflammation that is sustained by the release of oxidants in the alveolar space. The resulting increase in the expression of antioxidant enzymes, such as manganese superoxide dismutase and glutathione peroxidase, along with inducible nitric oxide synthase and activated signal pathway proteins, including MAPK/ERK kinase, then increases the expression of inflammatory cytokines (e.g., TNF-a, IL-1) and activates specific transcription factors (e.g., NF-κB, AP-1) [2].

In a previous study, when human lung epithelial cells were exposed to Cr(VI) *in vitro*, 150 genes were up-regulated and 70 genes down-regulated in high-density oligonucleotide arrays, indicating that Cr (VI) was involved in redox stress, calcium mobilization, energy metabolism, protein synthesis, cell cycle regulation and carcinogenesis in the cells [3]. Meanwhile, the exposure of cultured human peripheral lung epithelial HPL1D cells to nontoxic concentrations of Ni (II) acetate was found to influence the gene expression related to transcription, protein synthesis, cytoskeleton, signaling, metabolism, the cell membrane and extracellular matrix [4].

As *in vitro*, *in vivo* exposure or instillation exposure of various cell types to different metals and particulate matter, the effect of subchronic welding fume inhalation exposure on the blood *via* the lungs also result in similar gene profile changes as regards signal transductions including oncogenes and cell cycle-related genes, transcription factors, inflammatory response genes, xenobiotic metabolism-related genes, and stress-associated proteins [5].

Thus, given the recent toxicogenomic results on the welding fume exposure of rodents, non-human primates and humans, it is important to review the toxicogenomics to improve the current understanding of the welding-fume-induced gene expression profile between species. Accordingly, this review compares the toxicogenomic data obtained for rodents, non-human primates and welders from the perspective of toxicogenomic profiling for risk assessment of welding fume exposure.

Toxicogenomics of Rats Exposed to Welding Fumes

For the early detection of welding-fume-exposure-induced pulmonary fibrosis, the gene expression profiles of peripheral mononuclear cells from rats exposed to welding fumes were studied using suppression-subtractive hybridization (SSH) and a cDNA microarray. The Sprague-Dawley rats were exposed to stainless steel arc welding fumes for 2 h/day in an inhalation chamber with a 107.5 ± .6 mg/m^3 concentration of total suspended particulate (TSP) for 30 days. The total RNA was extracted from the peripheral blood mononuclear cells, the cDNA synthesized from the total RNA using the SMART™ PCR cDNA method, and SSH performed to select the welding-fume-exposure-regulated genes. The cDNAs identified by the SSH were then cloned into a plasmid miniprep, sequenced, and the sequences analyzed using the NCBI BLAST program. In the SSH cloned cDNA microarray analysis, five genes showed an increased expression of 1.9 fold or more, including *Rgs 14*, which plays an important function in cellular signal transduction pathways. Meanwhile, 36 genes showed no change in expression and 30 genes decreased their expression by more than 59%, including genes associated with the immune response, transcription factors, and tyrosine kinases. Among the 5,200 genes analyzed, 256

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genes (5.1%) were found to increase their gene expression, while 742 genes (15%) decreased their gene expression in response to the welding-fume exposure when tested using a commercial 5.0k DNA microarray. Therefore, unlike exposure to other toxic substances, prolonged welding fume exposure was found to substantially down-regulate many genes [5].

Using lung samples, well-annotated by histological observation and biochemical analysis, the gene expression profiles were examined to identify phenotype-anchored genes related to lung inflammation and the repair phenomenon after recurrent welding fume exposure. 7 genes (*Mmp12, Cd5l, Loc50101, Loc69183, Spp1, and Slc26a4*) were found to be significantly up-regulated according to the severity of the lung injury. In addition, the transcription and translation of *Trem2*, which was up-regulated in response to the repair process, were validated using a real-time polymerase chain reaction (PCR), Western blotting and immunohistochemistry. As such, this comprehensive and integrative analysis of the transcriptional changes that occur during repeated exposure provides important information on the inflammation and repair processes following welding-fume-induced lung injury [6]. It was found that the transcriptional levels of specific genes, including *Mmp12*, *Cd5l*, *Spp1* and *Slc26a4*, increased significantly (8 to 80 fold) according to the severity of the lung injury. This suggests that the genes identified here may serve as sensitive genomic biomarkers for lung injury induced by various factors including welding fume exposure. However, the *Trem2* gene was persistently up-regulated in both the exposure and recovery groups, and did not show differential expression according to the severity of the lung injury.

At the translational level, Trem2 was also over-expressed in the 2nd exposure and 2nd recovery groups, which was consistent with the microarray analysis. The Trem (triggering receptor expressed in myeloid cells) family, including Trem1 and Trem2, are cell surface receptors of mononuclear phagocytes as well as myeloid cells, and known to play a role in innate immunity and bacterial functions [7].

Their function and expression have already been studied in mice models and humans, yet investigations associated with lung inflammation and their expressions in rat models are limited. It has been reported that treating macrophages with an agonistic Trem1 antibody induces the activation of recognition receptors, such as the TLR or NLR family, and increases cytokine and chemokine secretion [8,9]. These studies also indicated that Trem1 is a positive regulator of inflammatory response.

In the case of Trem2, Hamerman et al. [10] reported that silencing Trem2 using shRNAi in bone-marrow-derived macrophages increased the secretion of the cytokine Tumor Necrosis Factor (TNF).

In addition, Trem2 overexpression in microglia leads to less TNF and inducible nitric oxide (iNOS) [11]. Thus, even though the signal pathway of Trem2 and its function are still unclear, Trem would would appear to be a negative regulator of inflammation. In this study, *Trem2* expression was not indicative of the severity of the lung injury, yet its persistent upregulation seemed to indicate that it plays an important role in recovery. Therefore, Trem2 expression may serve as a biomarker of the repair process coincidently occurring in the lungs during inflammation after welding fume exposure.

A toxicological function analysis showed that the genes belonging to the acute and prolonged response groups were actively involved in cellular movement and antigen presentation, whereas genes involved in cellular maintenance were predominantly identified in the prolonged response group. In addition, genes related to cell growth and proliferation was identified in the acute and prolonged response groups. Within the category of cellular function and maintenance, most of the genes identified were involved in phagocytosis or engulfment (*Asap2*, *Capg*, *Cd14*, *Cd44*, *Cd93*, *Fcgr2a*, *Icam1*, *Itgb2*, *Itgb5*, *Klf2*, *Trem2*, and *Un13d*), other genes related to cytoskeletal reorganization (*Ccl3*, *Cd44*, *Eng*, *Icam1*, *Trem2*, and *Vegfa*) were also identified. These results demonstrate that welding fume exposure exacerbates lung inflammation, as indicated by increased immune cell infiltration and antigen presentation. Moreover, as the welding fume particles were not completely removed, these processes continued throughout the recovery period as part of the prolonged immune response.

A repeated welding fume exposure/recovery model was developed to identify the phenotype-anchored gene sets that may serve as biomarkers for welding-fume-induced lung injury. An integrative genomic analysis demonstrated the activation of both inflammation and repair processes in the lungs when the rats were exposed to welding fumes, and several genes were identified as differentially expressed according to the severity of the lung injury, where one such gene, *Trem2*, may serve as a valuable biomarker for welding fume exposure (Table 1). In addition, pathway analyses provided insight into the sophisticatedly regulated transcription of these gene sets in the lungs and will aid in understanding the mechanisms of welding-fume-induced lung injury [6].

It has also provided a model of tumor necrosis factor-alpha-mediated pulmonary inflammation, fibrosis in normal adult lung, and it suggests that the fibrogenesis may be mediated by the secondary up-regulation of transforming growth factor-beta1 and induction of pulmonary myofibroblasts [12].

The transcriptional changes of 13 genes that were highly altered by treatment were confirmed by quantitative real-time PCR. Notably, *Mmp12*, *Cd5l*, *Ccl7*, *Cxcl5*, and *Spp1* related to the immune response were up-regulated only in the exposure group, whereas *Trem2*, *IgG-2a*, *Igh-1a*, and *Igh* were persistently up-regulated in both the exposure and recovery groups. In addition, several genes that might play a role in the repair process of the lung were up-regulated exclusively in the recovery group [13]. Collectively, these data may help elucidate the molecular mechanism of the recovery process of the lung after welding fume exposure.

These data suggest that findings in the animals' lung tumor model will have direct relevance to humans and these studies will serve as an initial step toward further characterization of the carcinogenic potential of welding fumes. Animal studies are certainly needed to determine if exposure to welding fume alone causes lung cancer. Based on the evidence in these studies, it cannot be concluded that welding fume induces neoplastic changes in the lungs of these animals, and these findings support the need to further investigate welding fumes as a possible carcinogenic particulate.

Toxicogenomics of Non-Human Primates Exposed to Welding Fumes

Cynomolgus monkeys were exposed to stainless steel manual metal arc (MMA-SS) welding fumes for 229 days and allowed to recover for 153 days. After the exposure and recovery period, the gene expression profiles were investigated using Affymetrix GeneChip® Human U133 plus 2.0. In total, it was confirmed that 1,116 genes were up or downregulated (over 2-fold changes, $P<0.01$) for the low concentration (31.4 ± 2.8 mg/m^3) and high (62.5 ± 2.7 mg/m^3) dose groups.

Functional analysis identified genes involved in immunological

	Up-regulated	Down-regulated
Rat	SAP kinases, thrombin receptors, and Gn-RH receptors. Inflammatory response genes (angiotensinogen and MHC class Ib). Xenobiotic metabolism-related genes (cytochrome P450 PB1, Y-b3 glutathione-S-transferase, betaine, and homocystein methyltransferase). *Mmp12*, *Cd5l*, *Loc50101*, *Loc69183*, *Spp1*, and *Slc26a4* were similarly or more markedly upregulated in exposure group. *Trem2* expression was elevated in recovery group.	Genes associated with signal transduction, cell growth, differentiation, transcription factor, and protein synthesis. Cathepsin E, dipeptidase, aflatoxin B1 aldehyde reductase, peroximal membrane protein 1, and NAD isocitrate dehydrogenase. Gene of stress-related proteins (2,4-dienoyl-CoA reductase precursor, thioredoxin reductase (TrxR2), glucocorticoid-regulated kinase, heat shock protein 70 (Hsp 70), and pyruvate carboxylase.
	The transcriptional changes that occur during repeated exposure provides important information on the inflammation and repair processes following welding-fume-induced lung injury.	
Non-human Primate	**With exposure:** Genes involved in signaling pathways (*DGKB*, *PIAS2*, *AXIN2*), metal ion binding (*TRIM2*), DNA binding (*HIST1*, *H2BC*), metabolism of immunological disease (*CHIT1*), genetic disorders, cancer, inflammatory diseases, cellular growth, proliferation, and development (*CHI3L1*, *RARRES1*, *DDHD1* and *CTSB*). **With recovery:** Genes involved in tRNA aminoacylation (*IGL@*, *TARS*), antigen presentation, or immune response (*HLA-DPB1*, *IGHM*, *GAGE12F*), cell differentiation or development (*THOC5*, *FNDC3A*, *DOCK7*), metabolism (*CHIT1*, *CPT1A*), and apoptosis (240890_at, *JAK2*). Genes involved in cancer, immunological diseases, and inflammatory diseases ranked high, other genes involved in cellular growth, proliferation, and cell cycle were also observed. *PPID*, *CFLAR*, *CPT1A* and *INSR*.	Genes involved in transport (*ABCA13*, *STEAP2*, *KCNH2*, *KCNV1*), transcription (236231_at, *ZNF738*, *HEY2*), cell adhesion (*ACTN2*), rRNA processing (*ADAT2*), and protein binding (*SLITRK6*). *GM2A*, *GRAP*, *CYP1B1*, *PTGFRN ID4*, and *NRGN*. Genes involved in heat shock protein binding (*DNAJC6*, *NTRK2*, *DNAJC10*), signal transduction (*RGS4*), proteolysis (*DPP10*), antigen presentation (*HLA-DPA1*), cell cycle arrest (*GAS2L3*), transcription (*ZNF483*), and development (*RICTOR*). *KLKB1*, *ATM*, *RAG1*, *UBASH3A*, *IGKC,* and *PTPN22*. *CHI3L1*, *GM2A*, *RARRES1*, *CTSK*, *DDHD1,* and *CTSB*.
	It changed the genes involved in the G1/S transition of the cell cycle; TR/RXR activation and fibrosis were identified in both the exposure and recovery groups. It changed the genes involved in gene regulation mechanisms by peroxisome proliferation, RAR activation, and oxidative stress response mediated by NRF2 were identified in the recovery group. It provides important information on the inflammation and repair processes from lung injury.	
Human	Xenobiotic metabolism-related genes (cytochrome P-450 family 1 sub- family B polypeptide, glutathione *S*-transferase) Stress-related genes (heat-shock protein 90, paraoxonase) Cathepsin S as a cystein protease working in antigen presentation and autoimmunity. Apoptosis WT1 regulator, interleukin (IL) 3 receptor alpha, cysteine sulfinic acid decarboxylase, and coronin.	Tumor necrosis factor (TNF)-alpha gene, which is known to increase its expression with short-term welding fume exposure *in vitro*, was essentially unchanged. Genes that decreased the most were synuclein beta, ribonuclease H1, angiotensin receptor, and glycosyltransferase.
	Most of these genes were disease- and immunity-related genes functioning for elimination of toxic material and disease reduction.	

Table 1: Toxicogenomic classification of significantly changed genes according to function following welding fume exposure.

disease in both groups. The differentially expressed genes in common between the monkeys and the rats following welding fume exposure were compared using the microarray data, and the gene expression of the selected genes was verified using a real-time PCR. The *CHI3L1, RARRES1, and CTSB* genes were up-regulated, while the *CYP26B1, ID4, and NRGN* genes were downregulated in both the monkeys and the rats following welding fume exposure. This is the first comprehensive gene expression profiling related to welding fume exposure in monkeys, and is useful for understanding the transcriptional changes in non-human primate lungs from welding fumes [14].

Actually, almost no genes were consistently expressed in both the rat lungs and the rat blood after welding fume exposure [15]. In addition, the use of a rodent model to predict the toxic effects in humans has inherent limitations due to interspecies differences in toxicological responses, although the central physiological functions are assumed to be essentially the same among mammals. From these reasons, the monkey model was used to investigate the gene expression profiling after welding fume exposure.

While the exposure concentrations of welding fumes were very similar in each study, the duration of the welding fume exposure differed between the monkey and rat models to allow the gene expression profiling of the monkeys to reflect and predict the molecular mechanism underlying welding fume exposure and recovery process in humans.

In the microarray analysis, the top-ranked differentially expressed genes involved in the inflammatory response were not primarily identified in the monkey lung exposure and recovery groups, which differed from the many immune response genes identified in the rats investigated previously [15]. However, a biofunctional analysis of all of the differentially expressed genes showed that about 50 genes identified in the exposure and recovery groups, respectively, appeared to be primarily involved in immunological disease. Table 1 shows the top-regulated genes related to welding fumes in the exposure and recovery groups. Based on a comparison of the expression changes for a total of 50 genes related to inflammation in the exposure and recovery groups, approximately 50% of the genes in the high dose group (62.5 ± 2.7 mg/m^3) were consistently up or down-regulated in both the exposure and recovery groups. This result suggests that even when a significant inflammatory response did not occur in the lungs of the welding fume exposed monkeys, the inflammatory response progressed concurrently during the recovery period.

Interestingly, there was a greater up-regulation of genes related to immunological disease in the recovery group than in the exposure group. The histopathology as well as the gene expression analysis revealed that the welding fumes were not removed during the recovery period; the inflammatory response consistently progressed during the recovery period.

Among the commonly deregulated genes in the monkeys and rats after welding fume exposure, the *CHI3L1, CTSK, CTSB* genes were upregulated, whereas the *GRAP, CYP1B1, CYP26B1 and ID4* genes were downregulated, and the transcriptional alterations were also confirmed by a real-time PCR. The transcriptional expression of *CHI3L1* is regulated by TNF or IL1B and CHI3L1, which are involved in macrophage differentiation [16]. CHI3L1 may play an important role in the early immune response in both monkeys and rats after exposure to welding fumes. Cathepsin K (CTSK), which is expressed in breast cancers, is also involved in the dendritic cell or macrophage

signaling pathway and is also associated with differentiation in a leukemia cell line [17]. Additionally, *CTSB* (cathepsin B gene), which was up-regulated during the welding fume exposure, is associated with apoptosis and proliferation in various cell lines, including lung cancer and fibroblast cell lines [18]. *GRAP*, which was down-regulated during the welding fume exposure, plays a role in negatively regulating the proliferation of lymphocyte interleukin-2 induction [19].

It was also found that *CYP1B1* and *CYP26B1* were deregulated in the lungs after exposure. In contrast, ID4, a transcriptional regulator and inhibitor of DNA binding, was downregulated during exposure. ID4 plays an important role in the differentiation and proliferation of neural cells and epithelial cell lines [20], yet its involvement in lung injury and lung inflammation has not been reported.

The *MMP12* gene was not differentially expressed in the monkey lungd, yet *MMP9* was upregulated, although its expression was not altered in the rats. *TREM2* was consistently up-regulated in both the monkeys and the rats, yet *TREM2* was excluded from the gene list, as its gene symbol did not match during the analysis. A previous study showed that *MMP12* was sensitively and significantly upregulated by exposure. This difference in gene expression may have been due to the degree of lung injury induced by the welding fumes or to interspecies variability [14].

This result also suggests that *TREM2* plays an important role in the lung injury induced by welding fume exposure in both monkeys and rats. Gene expression changes of these cytokines also indicate that the lung injury chronically progressed, even through the recovery period. Several genes involved in inflammatory response and proliferation were commonly deregulated in both the monkeys and the rats after exposure. This information could help understand the mechanisms in the lung tissue after welding fume exposure [14,21].

Toxicogenomics of Humans Exposed to Welding Fumes

A toxicogenomic chip developed to detect welding-related diseases in rats was validated for field trials. White blood cells or whole blood was purified and characterized from 20 subjects in the control group (average work experience of 7 yr) and 20 welders in the welding fume exposed group (welders with an average work experience of 23 yr). 253 rat genes homologous to human genes were obtained and validated using this chip, and a human cDNA chip spotted with 8,600 human genes was also used to detect any increased or decreased levels of gene expression among the welders.

After comparing the levels of gene expression between the control and welder groups using the toxicogenomic chips, 103 genes were identified as likely to be specifically changed by welding fume exposure. 18 of the 253 rat genes were specifically changed in the welders, while 103 genes from the human cDNA chip were specifically changed. The genes specifically expressed by the welders were associated with inflammatory responses, toxic chemical metabolism, stress proteins, transcription factors, and signal transduction. In contrast, there was no significant change in the genes related to short-term welding fume exposure, such as *TNFα* and *IL* [22].

In this study, the cDNA microarray confirmed its usefulness for the early detection of welder's pneumoconiosis. The changes found in the gene expression were related to inflammatory responses, xenobiotic metabolism; stress associated proteins, transcription factors, and signal transduction, while none of the short-term exposure specific genes (e.g., *TNFα* and *IL1*) were significantly increased.

To identify changes in the gene expression in the airways of 25 male, non-smoking welders, with and without lower airway symptoms, samples were compared before and after exposure. The microarray analysis resulted in several functional annotation clusters, where the highest enrichment score contained 'response to wounding', 'inflammatory response', and 'defense response'. Exposure to welding fumes changed the gene expression in the lower airways as regards genes involved in inflammatory and defense responses. Thus, the microarray and qPCR technique were able to reveal markers of exposure to welding fumes and possible disease-related markers. However, further studies are needed to verify the genes involved and to further characterize the mechanism for welding-fume-associated lower airway symptoms [23].

Exposure to welding fume nanoparticulate matter in humans is associated with inflammatory cytokine increases in the bronchoalveolar lavage fluid [24]. Rats exposed to welding fumes have shown marked pulmonary inflammatory responses and lipid peroxidation indicative of oxidative stress [25]. In addition, epithelial cells exposed to welding fumes or the transition metals associated with them exhibited oxidative stress which caused MAPK-dependent NF-κB and AP-1 activation leading to IL-8 up regulation [26]. Thus, the soluble transition metals appear to be the primary mechanism of oxidative stress and inflammation [27].

Hull and Abraham examined cases of aluminum-welding particle-induced pneumoconiosis, and found areas of severe dense fibrosis which were interspersed with macrophages containing particles [28]. The understanding of possible adverse health effects of exposure to welding fumes is essential to risk assessment and the development of prevention strategies and will impact a large population of workers [29]. Table 1 show the summary of this review classified each subject of which specific genes related to the exposure of welding fumes.

Discussion and Prospect

Gene-environment interactions contribute to complex disease development. The environmental contribution, in particular low level and prevalent environmental exposure, can constitute much of the risk and contribute substantially to disease. With the recent recognition that toxicological approaches more predictive of the effects in humans are required for risk assessment, *in vitro* human cell line data as well as animal data are being used to identify toxicity mechanisms that can be translated into biomarkers relevant to human exposure studies. Therefore, this review explored whether data from toxicogenomic studies of exposed welders can inform risk assessment by generating biomarkers of exposure, early effect, and/or susceptibility, elucidating the mechanisms of action underlying exposure related disease, and detecting response at low doses.

Jönsson et al. [23] showed a response to welding fumes by changes in gene expression associated with inflammation and defense. Furthermore, they indicates changes in *CSF3R*, *MMP25* and *TNFAIP6* during exposure to welding fumes among asthmatic compared to non-symptomatic welders, but this needs to be confirmed. The strengths with this study include the analysis of cells in the target tissue and not in proxy cells, e.g. from blood. Earlier studies have shown a large difference in gene expression in lungs compared to blood in rats after exposure to welding fumes [5,6].

The number of participants in these studies varies between 22 and 40, which is in the similar size as in Rim et al. and Wang et al. studies [22,30]. Rim et al. [22] found differences in gene expression among welders with pneumoconiosis and long-term exposure to welding fumes. They found differences in genes related to inflammatory responses,

xenobiotic metabolism, stress-associated proteins, transcription factors and signal transduction. Similar with these results, Jönsson et al. [23] observed differences in response to inflammation, when analyzing welding fumes-associated differences in gene expression.

Wang et al. [30] analyzed non-smoking welders for changes in gene expression in blood due to welding fume exposure for 5 h, using a self-controlled study design and clustering analyses. They found several biological processes that were significantly enriched which were: taxis, chemotaxis, defense response, inflammatory response, response to wounding and cell cycle. It is clear from these studies that several cell processes are involved during exposure and that the results are influenced by study design, cell types analyzed and length of exposure. These results indicate increased levels of *CSF3R*, *MMP25* and *TNFAIP6* during exposure to welding fumes among asthmatic subjects compared to non-symptomatic subjects, although the differences did not reach statistical significance.

In two studies of asthmatics, an increase in *TNFAIP6* was noticed in bronchial epithelium cells and bronchoalveolar lavage fluid (BALF) after allergen challenge [31,32]. This is in accordance with the results from our study. The roles of *TNFAIP6* in the airways during inflammation are likely to be diverse. *TNFAIP6* enhances the antiprotease activity of inter-α inhibitor against tissue kallikrein (TK). TK is involved in vasodilatation, increased vascular permeability, bronchoconstriction and airway hyper responsiveness. Moreover, *TNFAIP6* could play a role in protecting the epithelial surface in the airways against oxidative insults.

Baines et al. showed increased levels of *IL1R2* and *MMP25* in induced sputum from asthmatic people, who had decreased the level of antioxidants in the diet. Furthermore, *IL1R2* and *MMP25* showed statistically significant correlation with the percentage of sputum neutrophiles, and the authors suggest that these genes, among others, play an important role in the development of neutrophilic airway inflammation. Also *CSF3R* may be involved in neutrophil activity in inflammation, but its roll in asthma is not yet studied. Despite the changes in neutrophil-associated markers, we could not demonstrate a statistically significant effect on IL8 expression in this study. It also showed a down-regulation of *GSTA1* and *GSTA2* in a microarray analysis of induced sputum from asthmatic people with low antioxidant diet. In accordance, a down-regulation of a different gene in the glutathione superfamily, namely *GSTP1*, was indicated in the present microarray analysis (data not shown) [33].

In these studies, it was showed that it is possible to analyze changes in gene expression in induced sputum from welders and that disease-associated markers may be identified. However, it is suggested that well-defined study groups are used to be able to detect differences associated with disease.

Especially, the exposure to Cr (VI) has been known for more than a century to be associated with induction of cancer in humans. Carcinogenicity requires massive exposures and is only encountered in well defined occupational settings. Several biomonitoring studies have reported mechanisms of metals-induced genotoxicity, mutagenicity and carcinogenicity including direct DNA damage [34], induction of DNA-protein crosslinks [35], chromosome aberrations [36] and micronuclei [37] by welding fumes. These findings indicate that exposure to welding fumes induces genotoxic effects, indicating a potential health risk for welders. Therefore, to ensure maximum occupational safety, biomonitoring is of great value for assessing the risk for welding workers. Since DNA damage and cellular death are considered to be prime mechanisms during chemical carcinogenesis, these data may be relevant in risk assessment for protecting human health and preventing carcinogenesis.

Toxicogenomic studies now need to be designed with sufficient power to detect the true effects of the exposure. As more studies are performed and incorporated into databases, such as the 'Comparative Toxicogenomics Database (CTD)' and 'Chemical Effects in Biological Systems (CEBS)', data can be mined for the classification of newly tested chemicals and investigating the dose-response and inter-relationship among genes, the environment, and disease using a biological systems approach [38].

However, in the environmental field, there is a lack of hard data, as in the quality now beginning to be gleaned from genetic studies. Thus, if there is to be any hope of untangling the complex web of risks behind chronic diseases, researchers need to develop an "exposome", a highly detailed map of environmental exposure that may occur throughout a lifetime, which can then be mapped onto the etiology of major illnesses, including cancer. Technological and analytical advances are helping to include environmental exposure in the genetic profile for predicting a person's disease risk with greater precision [39].

The analysis usually involves a wide array of bioinformatics and statistics [40], regularly involving classification approaches [41]. In the risk assessment of industrial chemicals, toxicogenomics is used to study the toxic effects in defined model systems in order to draw conclusions on the toxic risk to workers or the environment. Administrations, such as the EPA and Occupational Safety and Health Administration (OSHA) currently preclude making regulatory decisions based on genomic data alone. However, they do encourage the voluntary submission of well-documented, high-quality genomic data. Both agencies are also considering the use of submitted data on a case-by-case basis for assessment purposes (e.g., to help elucidate the action mechanism or contribute to a weight-of-evidence approach) or for populating relevant comparative databases by encouraging parallel submissions of genomic data and traditional toxicological test results [42].

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