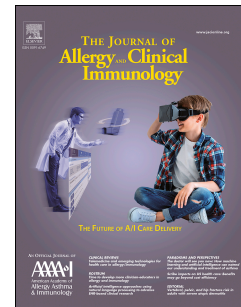


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Hille Suojalehto, MD, PhD, Joseph Ndika, PhD, Irmeli Lindström, MD, PhD, Liisa Airaksinen, MD, PhD, Piia Karisola, PhD, Harri Alenius, PhD



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Endotyping asthma related to three different work exposures

Hille Suojalehto MD, PhD¹, Joseph Ndika PhD², Irmeli Lindström MD, PhD¹, Liisa Airaksinen MD, PhD¹, Piia Karisola PhD², Harri Alenius PhD^{2,3}

¹ Occupational Medicine, Finnish Institute of Occupational Health, Helsinki, Finland

² Faculty of Medicine, Human Microbiome Research Program, University of Helsinki, Helsinki, Finland

³ Institute of Environmental Medicine (IMM), Karolinska Institutet, Stockholm, Sweden

Corresponding author:

Hille Suojalehto

Finnish Institute of Occupational Health Topeliuksenkatu 41 b, Helsinki

PO Box 40, FI-00032 Työterveyslaitos

Finland

Tel. +358 30 474 2545

hille.suojalehto@ttl.fi

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Abstract

Background. Work exposures play a significant role in adult-onset asthma, but mechanisms of work-related asthma are not fully elucidated.

Objective. We aimed to reveal the molecular mechanisms of work-related asthma associated with flour (FA), isocyanate (IA) or welding fume (WA) exposures and identify potential biomarkers that distinguish these groups from each other.

Methods. We used a combination of clinical tests, transcriptomic analysis and associated pathway analyses to investigate underlying disease mechanisms of the blood immune cells and the airway epithelium of 61 men.

Results. Compared to the healthy controls, the WA patients had more differentially expressed genes than the FA and IA patients both in the airway epithelia and in the blood immune cells. In the airway epithelia, active inflammation was detected only in WA patients. In contrast, large number of differentially expressed genes were detected in all asthma groups in blood cells. Disease-related immune functions in blood cells were suppressed in all the asthma groups including leukocyte migration and inflammatory responses and decreased expression of upstream cytokines such as TNF and IFN γ . In transcriptome-phenotype correlations, hyperresponsiveness ($R \sim |0.6|$) had the highest clinical relevance and associated with a set of exposure-group specific genes. Finally, biomarker subsets of only 5 genes specifically distinguished each of the asthma exposure group.

Conclusions. This study provides novel data on the molecular mechanisms underlying work-related asthma. We identified set of 5 promising biomarkers in asthma related to flour, isocyanate and welding exposure to be tested and clinically validated in future studies.

Capsule summary

Transcriptomic analysis of blood immune cells and airway epithelium revealed distinct profiles in asthma related to flour, isocyanate and welding fume exposure at work. Identified biomarkers could improve diagnostics of work-related asthma in the future.

Key messages

- Many differentially expressed genes were detected in blood immune cells of work-related asthma patients. Disease-related immune functions including leukocyte migration and inflammatory responses and decreased expression of upstream cytokines such as TNF and IFN γ were suppressed in these patients.
- In transcriptome-phenotype correlations, hyperresponsiveness had the highest clinical relevance and associated with a set of genes specific to asthma related to flour, isocyanate or welding fume exposure.
- We identified biomarker sets with 5 genes to each work-related asthma exposure groups capable to identify accurately these endotypes.

Keywords

Biomarkers, exposure, transcriptome-phenotype associations, work-related asthma

Abbreviations

BMI (body mass index), DEG (differentially expressed gene), FeNO (fractional exhaled nitric oxide), ICS (inhaled corticosteroid), IL (interleukin), IPA (Ingenuity's pathway analysis), IQR (interquartile range), PBMC (peripheral blood mononuclear cell), PCA (principal component analysis), WRA (work-related asthma)

Introduction

Asthma is a heterogeneous disease driven by interactions between airway epithelium, immune system and environmental exposure. It can be divided to several pheno- or endotypes based on clinical, functional and inflammatory features(1). Work exposures play a significant role in adult-onset asthma, 16% of all cases have been estimated to be attributable to workplace exposures(2). Work-related asthma (WRA) covers conditions which have symptoms and signs compatible with asthma and a relationship with exposures in the workplace(3). It encompasses subtypes of sensitizer and irritant induced occupational asthma and work-exacerbated asthma(4, 5). Diagnostic tests aim to identify these WRA subtypes, but in practice it can be difficult(6, 7).

Workplace exposures have airway effects through divergent mechanisms. Flours, like non-occupational protein allergens, act through type I hypersensitivity mechanisms(8). Isocyanates are widely used chemicals in the manufacture of polyurethane foams, sealants, elastomers, adhesives, and coatings. They act mainly via IgE-independent mechanisms which are not fully delineated, nonadaptive immune responses may be involved(8). Recent studies have revealed genetic variants of isocyanate-induced asthma and suggested contribution of TLR4(9, 10). The welding process releases potentially hazardous gases and fumes, mainly composed of metallic oxides, fluorides and silicates. These fumes include fine and ultrafine particles able to reach terminal bronchioles and alveoli. Inflammation, lung defense suppression, oxidative stress, DNA damage, and genotoxic effects have been observed after exposure to welding fumes(11). Exposure to welding fumes is common among WRA patients(12, 13) and has also been shown to increase the risk of COPD(14).

Linking pathobiological mechanisms to clinical variables of asthma such as age at onset, symptom triggers, lung function and inflammation type, is increasingly being used to identify asthma endotypes. When asthma endotypes are defined by distinct pathophysiological mechanisms, gene expression profiles of blood and nasal epithelia samples have shown promising results in identifying childhood asthma(15), mild-moderate/severe asthma(15, 16) and atopic/non-atopic asthma(17, 18). Asthma related to different well characterized workplace exposures comprise an interesting model to investigate asthma endotypes.

We aimed to evaluate the mechanisms of WRA by assessing clinical parameters and transcriptomic profiles in airway epithelium (nasal mucosa), and peripheral blood mononuclear cells (PBMC). We assessed WRA patients who were exposed to one of three workplace exposures (flours, isocyanates, or welding fumes) known to act via different mechanisms without categorizing them into subtypes based on clinical tests. We targeted to identify specific biomarkers for different exposure-related endotypes of WRA by combining clinical and transcriptomic findings.

Methods

Study population

Men aged 18-65 years were recruited. WRA patients were patients from the tertiary occupational disease clinic at the Finish Institute of Occupational Health during 2015-2018. Clinical assessment was carefully performed by a respiratory physician (HS, IL). All subjects were interviewed, and they filled a clinical questionnaire. Inclusion criteria were: 1) diagnosis of asthma, 2) exposure at work to flour, isocyanates or welding fumes, 3) emerging of asthma symptoms during work exposure and 4) aggravation of asthma symptoms during work

periods and relieve of symptoms during periods off work. Asthma diagnosis was verified from their medical files; they all showed reversible airway obstruction or bronchial hyperresponsiveness. WRA groups were: 1) flour asthma, FA, 2) isocyanate asthma, IA, and 3) welding asthma, WA.

The control group was recruited by using advertisements and social media. They did not have allergic symptoms related to common environmental allergens, asthma, chronic cough or dyspnea nor occupational exposure to flours, isocyanates or welding fumes.

The study was approved by the ethics committee of Helsinki University Central Hospital (291/13/03/00/2014). All participants signed their informed consent.

Sampling

Nasal biopsies and peripheral blood mononuclear cells (PBMC) were obtained from all subjects. The sampling of WRA patients was aimed within 24 hours after relevant exposure. None of the study participants had respiratory infection within 2 weeks prior to sampling and they were advised to stop nasal corticosteroid one month earlier when possible.

Inhaled steroid course for healthy controls

For safety reasons, inhaled corticosteroid (ICS) was not withdrawn from asthma patients before sample collection. To control the systemic ICS effect in the analysis, we assessed the ICS effect in the healthy controls. They used high dose ICS (budesonide DPI 800ug/day) for 5-6 weeks and a PBMC sample was taken before and after the treatment.

Figure E1 in the online data supplement shows a workflow outlining the allocation of the patient groups, sampling and analyses. The methods of clinical tests and transcriptomic analysis are described in the online supplement.

Data analysis

SPSS version 25.0 (IBM Corporation, Armonk, NY) was used for analysis of demographic and clinical parameters. Medians and interquartile ranges (IQR) were used to summarize continuous measures, while categorical variables were defined by their frequencies and proportions. Between group differences were analyzed using the Kruskal Wallis test for continuous variables and chi-squared tests for categorical variables. eUTOPIA bioinformatic packages(19), was used for transcriptomics analysis. After probe filtering and quantile normalization batch effects originating from labelling and array variance were removed (SVA-package with Combat function)(20). Differential analysis was performed by Limma Model analysis(21), using age, body mass index (BMI), smoking status and use of ICS as covariates where applicable. A fold change of 1.5 or greater and a Benjamini-Hochberg adjusted p-value less than 0.05 were considered as significantly differentially expressed genes (DEGs).

Clusters and heatmaps were generated using the Perseus program(22). K means clustering and principal component analysis (PCA) were used to visualize clustering of the gene profiles. The physiological implications of the DEGs was predicted via pathway enrichment analyses using Ingenuity's pathway analysis (IPA) tool (QIAGEN).

Results

Demographic and clinical characteristics

The study population comprised of 61 men: 46 WRA patients exposed to flours (FA, n=15), isocyanates (IA, n=20) or welding fumes (WA, n=11) and 15 healthy control subjects. In IA group 11 subjects were exposed to methylene diphenyl diisocyanate (MDI), 7 to hexamethylene diisocyanate (HDI) and 4 to toluene diisocyanate (TDI). In WA group all participants were exposed to stainless steel fumes in their workplace. Fifteen cases were diagnosed with occupational asthma. We did not detect statistically significant difference between the study groups in age, smoking status, BMI, or atopy (Table 1). Duration or frequency of work exposure, symptomatic work exposure, or asthma control did not differ significantly between the asthma groups. Work-related rhinitis was more common in the asthma groups than in the control group ($P<0.001$). Blood eosinophil count did not differ significantly between the study groups. Total IgE ($P=0.004$) and fractional exhaled nitric oxide (FeNO) ($P=0.011$) was highest in the FA group. During sampling 30 (65%) asthma patients used ICS, lung function did not differ significantly between the groups, and the asthma patients had more bronchial hyperresponsiveness than the controls ($P=0.004$). Objective tests showed association between asthma and work exposure (IgE-mediated sensitization to occupational allergen, suggestive workplace peak expiratory flow measurement or positive specific inhalation challenge) in 39 (85%) WRA patients (Table 1, Table E1).

Gene expression is significantly stronger, especially in nasal biopsies, in WA patients compared to FA and IA patients

Microarray-based transcriptomic profiling was carried out on RNA extracted from both nasal biopsies and PBMCs. The top two principal components, explaining 45% (nasal biopsy) and 59% (PBMC) of the variation between the samples, are shown in Figure E2. In the nasal biopsy samples, only individuals with WA clustered separately from controls (Figure E2A). All three WRA categories were visibly distinct from controls in the PBMC samples (Figure E2B). In order to identify disease-relevant genes for each exposure group, nasal biopsy and blood cell gene expression profiles of control subjects were compared to each of FA, IA and WA patients. Differential gene analysis was adjusted for age, BMI, smoking status and ICS use. The number of DEGs was clearly highest in WA patients (Figure 1A). In nasal biopsies, only 1 DEG was identified in FA group, 24 DEGs in IA group and 664 DEGs in WA group compared to controls (Figure 1B). In PBMC, FA patients exhibited 692 DEGs, IA patients 585 DEGs and WA group 1544 DEGs compared to controls (Figure 1C). Taken together, a much larger number of DEGs were detected in each WRA exposure group in PBMC in comparison to nasal biopsies.

Disease-related immune functions are suppressed in all asthma exposure groups in PBMC but enhanced in WA patients in nasal biopsies

To gain further insight into the underlying mechanisms of disease, all identified DEGs were subjected to functional enrichment analysis. Although Venn comparisons of significantly enriched diseases and biofunctions revealed unique disease mechanisms for each asthma exposure group (Figure 2A-B), the most significant gene functions were shared by IA and WA (nasal biopsy DEGs) and between FA, IA and WA (PBMC DEGs). In nasal biopsy, the top significant functions common to the IA and WA categories were; *quantity of antigen presenting cells* and *quantity of leukocytes* (Figure 2A). In blood cells, genes involved in *neutrophil degranulation*, *leukocyte migration* and *general inflammatory response*, were identified as the top enriched functions common to all asthma groups (Figure 2B). A heatmap based on significantly enriched pathways, separates nasal biopsy from blood cell sample groups, and further substantiates WA as the most pathologic asthma group in this cohort

(Figure 2C). Similar to the gene expression profiles, significance of enriched disease or biofunctions was greater in PBMC than in nasal biopsy (Figure 2C).

Ranking of the top 20 enriched functions and a hierarchical cluster of their predicted activation status is shown in Figure 3A and 3B respectively. Genes involved in *leukocyte migration*, *degranulation of neutrophils* and general *inflammatory response*, were highly represented (FDR $1E-58.1$ to $1E-15.6$) in DEGs from peripheral blood cells of FA, IA and WA groups (Figure 3A). DEG in nasal biopsy of WA patients are represent the same diseases and biofunctions as the DEG identified in the blood cells of FA, IA and WA patients. However, whilst the underlying pathways are predicted to be activated in the nasal biopsy gene profile of FA, IA and WA patients, the same pathways are predicted to be inactivated in the PBMC of WA patients (Figure 3B).

We next sought to answer whether some of these DEGs are under the control of upstream inflammatory regulators that play important roles in immune system disorders. The top 20 upstream regulators predicted in the IPA analysis are shown in Figure E3A. These regulators include the proinflammatory cytokines TNF, $IFN\gamma$, $TGF\beta 1$, $IL1\beta$, CSF2, as well as anti-inflammatory cytokines such as IL13, IL4, IL6 and IL10. Yet again, whilst the nasal biopsy gene expression profile was consistent with activation of these cytokines, the PBMC gene expression profile revealed suppression or inactivation of these same cytokines (Figure E3B). The trend in expression of selected upstream regulators is in line with the activation states predicted from the expression profiles of their downstream targets (Figure E4).

Gene-phenotype correlations reveal genes with high clinical relevance

Hierarchical clustering based on Pearson's correlation coefficient, revealed two distinct clusters of clinically relevant disease features (Figure E5). Clinical measures of lung function, eosinophilic inflammation, bronchial hyperresponsiveness, atopy and asthma control make up cluster 1, while demographic parameters and features related to disease severity and exposure (age, smoking, BMI, ICS dose, frequency of exposure, duration of exposure and duration of symptoms) make up cluster 2 (Figure E5).

We next sought to answer whether the expression of any disease-relevant genes identified from PBMC transcriptome profiling, correlates with specific diagnostically relevant clinical features in cluster 1. In all three asthma groups, genes with a Pearson's correlation coefficient, $-0.6 \leq R \leq 0.6$, to at least 1 clinical feature were included. A heatmap illustrating correlations of DEG to diagnostically relevant clinical criteria in each patient groups are shown in Figure 4. The correlated genes and their corresponding correlation coefficients are provided in the supplementary Table E2. PCA analysis shows that the expression profile of these highly correlating genes clustered the patients separately from the controls in all asthma groups (Figure 4A-C, lower panel). Bronchial hyperresponsiveness had the strongest correlation to gene expression for all asthma groups (Figure 4A-C). Gene correlations to all other clinical parameters were weak or non-existent, except for specific IgE (S-IgE) and total IgE (T-IgE) which had the strongest correlation to gene expression in the FA group (Figure 4A).

Similarly, we identified several differentially expressed genes in the PBMC associated ($-0.6 \leq R \leq 0.6$) to at least one parameter from cluster 2. A heatmap of the correlated genes for each WRA exposure group is depicted in Figure E6A. The strongest modulators of gene expression amongst WRA patients were, ICS use in all WRA subtypes, exposure frequency in IA, smoking in IA and WA subtypes and age, exposure duration, symptom duration, and BMI in the FA subtype. The top enriched biological processes represented by patient

demographics associated DE genes in all WRA subtypes was *regulation of cytokine production* (Figure E6B).

Biomarker sets with only 5 genes accurately identify work-related asthma exposure-related endotypes

We next sought to identify endotype biomarkers that could facilitate the diagnosis by identification of group-specific DEGs that are associated with clinical features. In Figure 4, 302, 189 and 67 genes are associated to at least 1 clinical feature in FA, IA and WA patients, respectively. A Venn comparison of these clinical-feature-associated DEGs is shown in Figure E7A and the most significant pathways represented by these genes are shown in Figure E7B. Top enriched disease or functions identified by these genes are *quantity of myeloid cells and cellular infiltration by leukocytes* in FA patients, *degranulation of immune cells* in IA patients, and *quantity of immune cells* in WA patients. To further refine the diagnostic potential Venn comparisons were used to identify unique DEG that are associated to diagnostically relevant clinical features. This way we end up with genes that are both associated to at least one clinical feature and uniquely differentially expressed in that particular asthma group. Unique DEG (Figure 1C above), were 42, 36 and 734 genes in the FA, IA and WA endotypes, respectively. The expression profile of these asthma-exposure-group-specific DEG in PBMCs, separates all patients from controls (Figure E7C). Separation of patient from control samples was most distinct in the FA and WA groups. In total, 24, 9 and 49 of such genes were identified from the FA, IA and WA groups, respectively (Figure E7D). We then calculated the intraclass correlation coefficients for each of these genes (Table E3).

The PCA plots based on the expression profile of the top 5 genes for each asthma exposure groups is shown in Figure 5. We propose these top 5 genes as biomarker panel candidates for flour-associated asthma (Figure 5A), isocyanate-associated asthma (Figure 5B) and welding-associated asthma (Figure 5C). These biomarkers are: downregulation of *MYOM2*, *CDK5R1*, *ZDBF2*, *VCPIP1* together with upregulation of *ARPC5L* to FA, downregulation of *TST*, *TMEM55A*, *SPHAR* and *DNAJA1* together with upregulation of *ANO6* to IA and downregulation of *ITGA5* together with upregulation of *HERC2P4*, *DHRS3*, *NUCB2* and *DSC1* to WA.

Discussion

The mechanisms of WRA are not fully elucidated and disease endotype-specific biomarkers are not currently known. We used a combination of clinical tests and transcriptomic analysis of blood immune cells and airway epithelium to investigate underlying disease mechanisms and to identify endotype-specific biomarkers. Elaborating on pathological mechanisms and identifying disease associated biomarkers in WRA related to different exposures, have potential to improve diagnosis and facilitate the development of targeted treatment.

We studied WRA patients with exposure to three major workplace agents associated with different disease mechanisms— flour, isocyanates or welding fumes. WRA can be related to various other workplace exposures, and our results cannot be generalized to WRA related to other exposure types(3). The WRA patients were recruited from tertiary occupational medicine clinic, in which patient population is preselected to have high probability of occupational disease. WRA patients were not categorized into clinical subgroups of WRA (occupational asthma, work-exacerbated asthma). Clinical tests targeted to diagnose occupational asthma are typically aimed to detect sensitizer induced asthma, whereas welding

fumes act via different mechanisms(11). These tests may select cases based on disease mechanisms, give false negative results or cannot distinct reliably occupational asthma from work-exacerbated asthma(6, 7) . We studied WRA without preselection based on clinical test and used same inclusion criteria in all exposure groups (asthma, emerging and aggravation of symptoms when exposed to specific occupational agents). Nevertheless, we detected biomarkers that identify each WRA exposure group, supporting homogeneity and similar background mechanisms among participants in each group. Overall, our results enlighten mechanisms of asthma related to certain work exposures rather than mechanisms of WRA categories (occupational or work-exacerbated asthma), and the results cannot be applied in the differential diagnosis of them. All WRA patients fulfilled diagnostic criteria of asthma prior to sampling. At the time of sampling, most of them used ICS and clinical tests showed mainly well or partly controlled asthma.

To diminish heterogeneity of the study population, all participants were male and WRA patients were currently exposed to the relevant occupational agent. Demographic variables did not differ between the groups. ICS treatment had great effect on transcriptome in previous studies(23), in order to avoid bias related to ICS, steroid effect was examined in healthy controls and taken into account in the analysis. Most WRA patients reported work-related rhinitis similar to previous studies(24, 25). Rhinitis is considered as a manifestation of united airway disease including both upper and lower airways, rather than a separate phenomenon(26). Similar to previous studies, asthma related to high molecular weight allergens (flour) showed IgE-mediated sensitization to the occupational agent and had high FeNO level reflecting T2 derived inflammation(24, 27) and represents T2 phenotype of asthma, whereas isocyanate asthma group was mainly not IgE-associated and T2 inflammation was not detected in isocyanate or welding related asthma groups.

We selected nasal epithelia as a local sampling site, because in previous studies the quality of nasal epithelia samples has been sufficient for transcriptomic analysis(28), whereas quality of sputum samples varies(29). Localized (nasal biopsy) and systemic (PBMC) gene expression was profiled after exposure to either flour, isocyanates or welding fumes. Several times higher number of DEG in blood immune cells relative to nasal biopsy suggests that the pathomolecular changes are predominantly existing in the systemic circulation at the time of sampling. Our results also suggest that ongoing inflammation in the airway epithelia in patients with flour or isocyanate asthma was virtually non-existing. It is possible, however, that the use of ICS may partially explain why inflammation in the airway epithelium could not be identified these WRA groups. In contrast, exposure to welding fumes was related to changes in the gene expression which was detectable both in the airway epithelia and in PBMC – indicative of hazardous inflammatory process in the airways. Based on extensive literature search, comparable comprehensive transcriptomics studies in human subjects are practically non-existing(30-33) and to our knowledge there are no comparable studies of WRA. A few well-designed studies have been reported in mice(34-37) and rats(38-40) but their relevance in the identification of disease endotype specific biomarkers in patients is questionable(41).

Considering the most robust results derived from the pathway enrichment analysis all asthma groups exhibited significant suppression of immune functions related to cell migration and cell movement in blood immune cells. Only welding related asthma patients demonstrated enrichment of cell migration related immune functions, but contradictory to PBMC, those pathways were enhanced in the airway epithelia. Similar phenomena could also be seen with predicted upstream regulators in welding-related asthma patients – several inflammatory cytokines (e.g. TNF, IFN γ , IL1 β , TGF β 1) were suppressed/inactivated in the blood immune cells but stimulated/activated in the nasal epithelia. This suggests that observed gene

expression changes, both in nasal epithelia and peripheral blood cells, in welding-related asthma patients are controlled by a central pathological mechanism. It can be speculated that these contradicting gene expression profiles are due to differences of the inflammatory cell pools in the nasal epithelium and in the circulation. In other words, inflammatory cells which are likely the main source of the exposure-related gene expression are recruited to the site of the inflammation, i.e. airway epithelia, and are therefore missing from the circulation. In the nasal biopsies, due to the limited number of identified DEGs, it is unsurprising that very few to none significantly enriched immune functions were identified from the flour and isocyanate asthma groups.

To identify genes with high clinical relevance, we carried out correlation analysis between DEGs identified in blood cells and several clinical parameters. Bronchial hyperresponsiveness showed the strongest correlation to gene expression in all asthma groups. Interestingly, FeNO and specific and total IgE demonstrated moderate correlations to gene expression in flour asthma but not in other groups. This is likely due to more prominent IgE-associated and T2-type immunity in flour asthma patients compared to isocyanate and welding related asthma groups which are more non-T2 associated asthma types. Identification of genes correlating with important clinical function may facilitate development of a molecular diagnostic tool for more accurate clinical diagnosis.

The influence of smoking status, subject age, BMI, ICS dose were adjusted during between group (patient vs control) differential expression analysis. However, within each WRA exposure group these factors, in addition to exposure duration and frequency, and symptom duration, were also found to affect expression of disease-related genes. The involvement of these genes in regulation of cytokine production, indicates that these factors may modulate disease related features by affecting the extent of pro-/anti-inflammatory signaling. This finding is consistent with the previous report that the severity asthma is associated with older age, higher BMI and higher rate of tobacco smoking(42) and severity of occupational asthma with persistent exposure to the relevant occupational agent and longer duration of symptomatic exposure(43). However, a larger cohort will be needed to fully unravel the mechanisms via which these factors influence disease features.

Despite the overlapping gene expression profiles between the WRA groups in the blood immune cells, indicative of similarities in the pathological mechanisms, disease specific differences were also found. In order to identify exposure-related specific biomarkers we combined and analyzed the information on the unique DEGs genes identified in PBMCs, as well as the DEGs that are associated to the clinical parameters. Our prioritization analysis revealed specific sets of 5 candidate genes for each exposure-related endotype capable of distinguishing it as a disease subgroup. Further studies in unrelated patient materials are needed to confirm and validate the predictability of our biomarkers.

Conclusion

This study enlightens asthma mechanisms related to three different types of exposures present at workplace. Active inflammation in airway epithelia could be detected from the gene expression only in welding-related asthma. In contrast, large number of differentially expressed genes were detected in all patient groups in the blood immune cells. Gene-phenotype correlations revealed genes with high clinical relevance – bronchial hyperresponsiveness was the strongest clinical feature correlating with gene expression. Our study identified biomarker sets capable to identify accurately asthma related to flour, isocyanates or welding. Our results could provide basis for future attempts to create diagnostic biomarkers for clinical settings.

388

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Figure legends

Figure 1. Overview and comparison of disease-relevant genes identified from nasal biopsy and blood cell transcriptome profiles of patients with asthma related to flour, isocyanates and welding fumes. The highest number of differentially expressed genes (DEG) were identified in patients whose asthma was related to welding fumes (A). With only 1 and 24 differentially expressed genes, the nasal biopsy transcriptomes of patients with flour and isocyanate asthma were quite similar to controls. Venn diagrams of DEG identified in nasal biopsy (B) and PBMC (C), suggest overlapping disease mechanisms across the different types of asthma in this cohort.

Figure 2. Gene function enrichment analyses. Venn comparisons of significantly overrepresented disease and biofunctions within differentially expressed genes identified in nasal biopsy (A) and peripheral blood mononuclear cells (PBMC) (B). Overlapping pathways in both tissue types correspond to most significantly enriched gene functions. A hierarchical cluster of all enriched pathways based on negative log of pathway false discovery rate (-log FDR) suggests greater similarity of disease mechanisms between flour and isocyanate asthma. Non-enriched pathways are shown in white, while the color gradient from blue through yellow to red, depicts increasing significance of the identified enriched disease and biofunction pathways. FA, flour asthma; IA, isocyanate asthma; WA welding asthma.

Figure 3. Comparative ranking (-log FDR) of topmost pathways enriched by differentially expressed genes across each asthma exposure group (A). Activity of each pathway was predicted using the activation Z-score. A heatmap of these pathways, based on their activation states is shown in (B). Only heat signatures of pathways with activation Z-scores exceeding significance threshold: ≤ -2 for suppression and ≥ 2 for activation, are shown in the heatmap. All functions that are suppressed in the PBMC gene profile, are predicted to be activated in nasal biopsy gene profile. FA, flour asthma; IA, isocyanate asthma; WA welding asthma.

Figure 4. Heatmaps of the correlation (Pearson) between differentially expressed genes (DEG) identified in blood cells of each asthma exposure group, and selected clinical features with relevance for asthma, in Flour/Control (A, upper panel), Isocyanate/Control (B, upper panel) and Welding/Control (C, upper panel). The clinical features correlated to identified DEG are fractional exhaled nitric oxide (FeNO), specific IgE (S-IgE), Total IgE (T-IgE), airway hyperresponsiveness (Hyperresponsiveness), blood eosinophil count (B-Eos) and lung function (FEV1/FVC & FEV1%). PCA plots based on genes with a correlation of $-0.6 < R < 0.6$, to at least one clinical parameter, is shown for each asthma group (A, B, and C, lower panel).

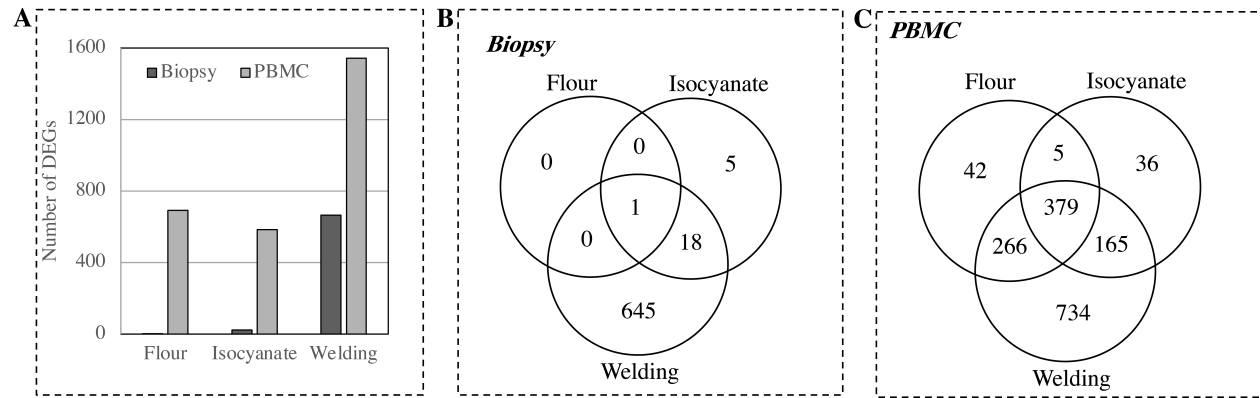
Figure 5. Exposure group specific biomarkers were identified as subsets of genes that are differentially expressed in only one asthma group, and whose expression profile correlates ($-0.6 < R < 0.6$) to at least one clinical feature. A PCA plot depicting asthma group separation from controls, based on the top 5 biomarker candidate differentially expressed genes specific to Flour asthma (FA) (A), Isocyanate asthma (IA) (B) and Welding asthma (WA) (C) is shown. From left to right, the best distinction between patients and controls is always observed for the asthma group from whence the potential biomarker genes were identified as uniquely differentially expressed and correlated to a clinical diagnostic feature.

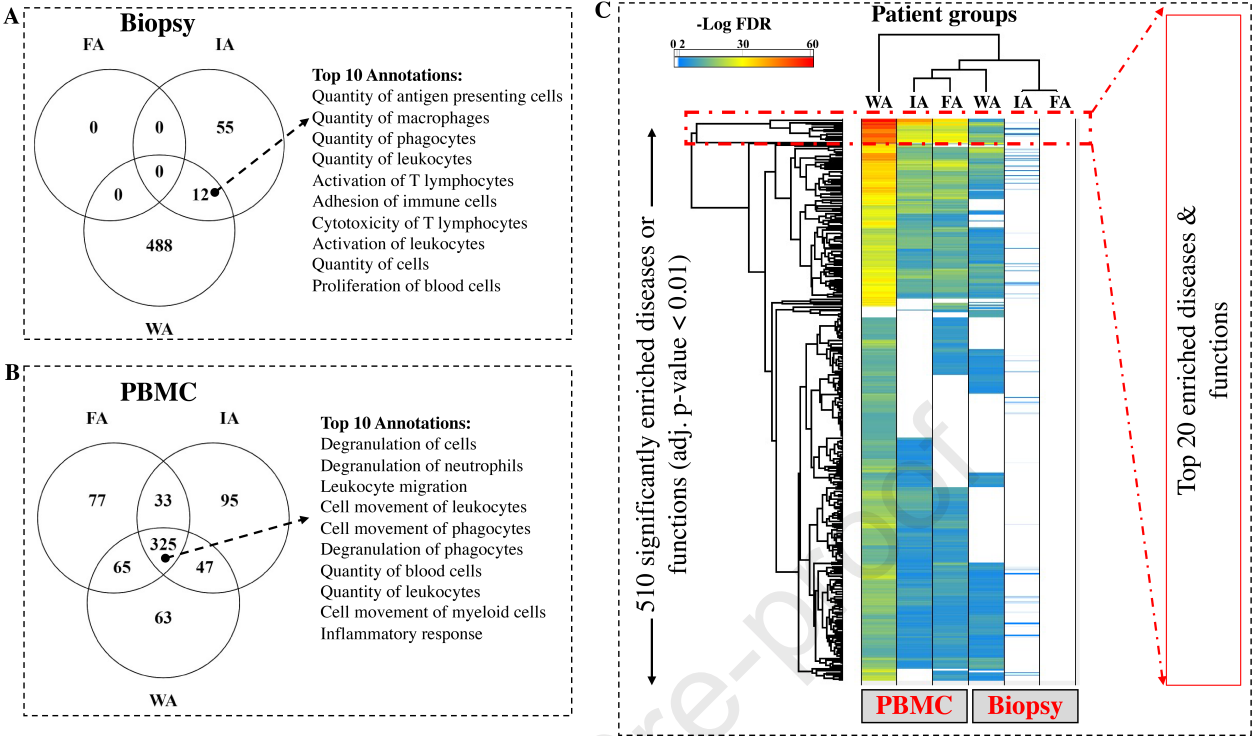
Table 1. Characteristics of 61 men participating the study. The tests were performed at the time of sample collection. Lung function tests confirming asthma diagnosis were performed earlier and not shown in the table.

	Asthma related to flour, FA (n=15)	isocyanates, IA (n=20)	welding fumes, WA (n=11)	Healthy controls (n=15)	P value
Age, years, md (IQR)	33.5 (27.0-49.4)	47.5 (29.5-54.2)	43.5 (37.8-53.2)	36.6 (34.9-47.9)	0.553
Current smoking, n (%)	1 (7)	2 (10)	2 (18)	2 (13)	0.820
Body mass index (BMI), kg/m ² , md (IQR)	25.6 (23.1-29.4)	28.4 (25.4-32.3)	27.9 (25.1-31.7)	25.7 (23.5-28.1)	0.074
Atopy ^a , n (%)	10 (67)	10 (50)	7 (64)	4 (27)	0.125
Duration of work exposure, years, md (IQR)	10.1 (6.8-30.0)	9.8 (1.9-19.4)	12.3 (8.0-20.0)	NA	0.531
Frequency of work exposure, n (%)					0.314
Daily $\geq$ 1 hour	13 (87)	11 (55)	9 (82)	NA	
Daily < 1 hour	1 (7)	2 (10)	1 (9)	NA	
At least one a week	1 (7)	5 (25)	0 (0)	NA	
Less than weekly	0 (0)	2 (10)	1 (9)	NA	
Duration of asthma symptoms at work, years, md (IQR)	2.0 (1.0-5.0)	1.0 (0.4-4.0)	4.0 (1.75-5.0)	NA	0.138
Asthma control test score, md (IQR) (n=42)	21 (16-24)	18 (15-23)	20 (17-23)	NA	0.560
Use of ICS within a month, n (%)	7 (47)	14 (70)	9 (82)	0 (0)	<0.001
ICS dose, n (%) ^b (n=30)					0.517
Low	0 (0)	1 (7)	0 (0)	NA	
Medium	6 (86)	7 (50)	6 (67)	NA	
High	1 (14)	6 (43)	3 (33)	NA	
Work-related rhinitis, n (%) (n=60)	12 (80)	12 (63)	8 (73)	0 (0)	<0.001

Nasal corticosteroid within a month	2 (13)	3 (15)	1 (9)	0 (0)	0.484
Total IgE, kU/l, md (IQR)	101 (63-498)	57 (24-141)	79 (23-207)	23 (6-34)	0.004
Blood eosinophils / μ l, md (IQR) (n=60)	210 (140-360)	220 (143-293)	140 (108-278)	160 (70-330)	0.538
FeNO, md (IQR)	39 (24-63)	19 (12-28)	18 (14-32)	15 (8-24)	0.011
FEV ₁ % pred, md (IQR)	80 (71-87)	87 (77-93)	91 (76-97)	86 (75-95)	0.160
FEV ₁ /FVC, md (IQR)	75 (66-79)	75 (72-77)	76 (70-78)	79 (74-81)	0.250
Nonspecific bronchial hyperresponsiveness, n (%)					0.004
Moderate	4 (27)	1 (5)	1 (9)	0 (0)	
Mild	8 (53)	9 (45)	4 (36)	1 (7)	
No	3 (20)	10 (50)	5 (46)	14 (93)	
Clinical tests show association between work and asthma ^c	15 (100)	16 (80)	8 (73)	NA	0.117

FA, flour asthma; FeNO, Fractional exhaled nitric oxide; FEV₁, Forced expiratory volume in one second; FVC, Forced vital capacity; IA, isocyanate asthma; ICS, inhaled corticosteroid; IQR, interquartile range; IgE, Immunoglobulin E; md, median; NA, not available; PEF, Peak expiratory flow; SPT, skin prick test; WA, welding asthma; ^aPositive SPT to at least one environmental allergen, ^baccording to Global Initiative for Asthma guidelines(44); ^cSpecific IgE or specific inhalation challenge to occupational allergen positive or workplace PEF suggestive for WRA.





A

Diseases & Biofunctions	Biopsy			PBMC		
	FA	IA	WA	FA	IA	WA
Leukocyte migration	0.0	0.0	24.0	33.7	36.7	58.1
Degranulation of cells	0.0	0.0	8.9	34.2	42.5	62.1
Degranulation of phagocytes	0.0	0.0	8.9	32.8	42.4	58.3
Degranulation of neutrophils	0.0	0.0	7.3	34.1	42.4	56.7
Cell movement of leukocytes	0.0	0.0	20.4	33.3	32.7	50.7
Systemic autoimmune syndrome	0.0	1.2	17.6	25.7	37.0	50.8
Quantity of leukocytes	0.0	2.9	17.9	30.8	26.6	49.7
Migration of cells	0.0	1.2	20.0	27.8	27.3	50.0
Cell movement of phagocytes	0.0	0.0	14.7	33.0	31.3	45.9
Quantity of blood cells	0.0	0.0	16.7	30.8	24.8	48.8
Cell movement of myeloid cells	0.0	0.0	15.4	30.6	28.6	45.3
Rheumatic Disease	0.0	0.0	16.9	27.4	28.5	44.3
Activation of leukocytes	0.0	2.1	12.0	24.7	25.1	52.3
Inflammatory response	0.0	0.0	15.6	27.9	25.1	44.9
Inflammation of joint	0.0	0.0	10.1	26.3	27.1	43.9
Quantity of phagocytes	0.0	2.9	17.5	26.5	23.8	35.3
Function of leukocytes	0.0	1.8	12.1	22.6	21.4	44.9
Immune response of cells	0.0	0.0	8.1	22.7	24.1	42.3
Cellular infiltration	0.0	0.0	12.3	24.8	23.1	35.6
Quantity of myeloid cells	0.0	0.0	15.2	26.8	18.1	35.5
negative log FDR (0 - 65)						

B