Endotyping asthma related to 3 different work exposures

Suojalehto, Hille

2021-10

Suojalehto , H , Ndika , J , Lindström , I , Airaksinen , L , Karisola , P & Alenius , H 2021 , ' Endotyping asthma related to 3 different work exposures ' , Journal of Allergy and Clinical Immunology , vol. 148 , no. 4 , pp. 1072-1080 . https://doi.org/10.1016/j.jaci.2021.07.019

http://hdl.handle.net/10138/352245
https://doi.org/10.1016/j.jaci.2021.07.019

Endotyping asthma related to 3 different work exposures

Hille Suojalehto, MD, PhD,[a] Joseph Ndika, PhD,[b] Irmeli Lindström, MD, PhD,[a] Liisa Airaksinen, MD, PhD,[a] Piia Karisola, PhD,[b] and Harri Alenius, PhD[b,c] *Helsinki, Finland, and Stockholm, Sweden*

Background: Work exposures play a significant role in adult-onset asthma, but the mechanisms of work-related asthma are not fully elucidated.
Objective: We aimed to reveal the molecular mechanisms of work-related asthma associated with exposure to flour (flour asthma), isocyanate (isocyanate asthma), or welding fumes (welding asthma) 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 the underlying disease mechanisms of the blood immune cells and the airway epithelium of 61 men.
Results: Compared with the healthy controls, the welding asthma patients had more differentially expressed genes than the flour asthma and isocyanate asthma patients, both in the airway epithelia and in the blood immune cells. In the airway epithelia, active inflammation was detected only in welding asthma patients. In contrast, many differentially expressed genes were detected in blood cells in all 3 asthma groups. Disease-related immune functions in blood cells, including leukocyte migration and inflammatory responses, and decreased expression of upstream cytokines such as TNF and IFN-γ were suppressed in all the asthma groups. In transcriptome-phenotype correlations, hyperresponsiveness ($R \sim |0.6|$) had the highest clinical relevance and was associated with a set of exposure group–specific genes. Finally, biomarker subsets of only 5 genes specifically distinguished each of the asthma exposure groups.
Conclusions: This study provides novel data on the molecular mechanisms underlying work-related asthma. We identified a set of 5 promising biomarkers in asthma related to flour, isocyanate, and welding fume exposure to be tested and clinically validated in future studies. (J Allergy Clin Immunol 2021;148:1072-80.)

Key words: *Biomarkers, exposure, transcriptome-phenotype associations, work-related asthma*

Asthma is a heterogeneous disease driven by interactions between the airway epithelium, the immune system, and environmental exposure. It can be divided into several phenotypes 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 having 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 doing so can be difficult.[6,7]

Workplace exposures have airway effects through divergent mechanisms. Flours, like nonoccupational protein allergens, act through type I hypersensitivity mechanisms.[8] Isocyanates are chemicals that are widely used in the manufacture of polyurethane foams, sealants, elastomers, adhesives, and coatings. They act mainly via IgE-independent mechanisms that are not fully delineated, and nonadaptive immune responses may be involved.[8] Recent studies have revealed genetic variants of isocyanate-induced asthma and suggested a contribution of Toll-like receptor 4.[9,10] The welding process releases potentially hazardous gases and fumes composed mainly of metallic oxides, fluorides, and silicates. These fumes include fine and ultrafine particles that are 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 patients with WRA,[12,13] and it has also been shown to increase the risk of chronic obstructive pulmonary disease.[14]

Linking pathobiologic 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 pathophysiologic mechanisms, gene expression profiles of blood and nasal epithelia samples have shown promising results in identifying childhood asthma,[15] mild-to-moderate or severe asthma,[15,16] and atopic or nonatopic asthma.[17,18] Asthma related to different well-characterized workplace exposures comprise an interesting model to investigate asthma endotypes.

From [a]Occupational Medicine, Finnish Institute of Occupational Health, Helsinki; [b]the Faculty of Medicine, Human Microbiome Research Program, University of Helsinki; and [c]the Institute of Environmental Medicine, Karolinska Institutet, Stockholm.

Supported by the Finnish Work Environment Fund (grant 114350).
Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.
Received for publication April 27, 2021; revised July 6, 2021; accepted for publication July 22, 2021.
Available online July 29, 2021.

Corresponding author: Hille Suojalehto, MD, PhD, Finnish Institute of Occupational Health Topeliuksenkatu 41 b, Helsinki, PO Box 40, FI-00032 Työterveyslaitos, Finland. E-mail: hille.suojalehto@ttl.fi.

0091-6749

https://doi.org/10.1016/j.jaci.2021.07.019

Abbreviations used
BMI: Body mass index
DEG: Differentially expressed gene
FA: Flour asthma
FENO: Fractional exhaled nitric oxide
IA: Isocyanate asthma
ICS: Inhaled corticosteroid
PCA: Principal component analysis
WA: Welding asthma
WRA: Work-related asthma

We aimed to evaluate the mechanisms of WRA by assessing clinical parameters and transcriptomic profiles in airway epithelium (nasal mucosa) and blood immune cells (PBMCs). We assessed patients with WRA who were exposed to 1 of 3 items encountered in the workplace (flours, isocyanates, or welding fumes) that are known to act via different mechanisms without categorizing them into WRA subtypes with clinical tests. We sought to identify specific biomarkers for different exposure-related endotypes of WRA by combining clinical and transcriptomic findings.

METHODS

Study population

Men aged 18 to 65 years were recruited. The study patients with WRA were patients from the tertiary occupational disease clinic at the Finish Institute of Occupational Health from 2015 to 2018. Clinical assessment was carefully performed by a respiratory physician (H.S. or I.L.). All subjects were interviewed, and they filled out a clinical questionnaire. The inclusion criteria were as follows: (1) diagnosis of asthma; (2) exposure to flour, isocyanates, or welding fumes while at work; (3) emergence of asthma symptoms during work exposure; and (4) aggravation of asthma symptoms during work periods and relief of symptoms during periods off work. Asthma diagnosis was verified from the participants' medical files; they all showed reversible airway obstruction or bronchial hyperresponsiveness. The WRA groups consisted of men with (1) flour asthma (FA), (2) isocyanate asthma (IA), and (3) welding asthma (WA).

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

The study was approved by the ethics committee of Helsinki University Central Hospital (approval no. 291/13/03/00/2014). All participants signed forms indicating their informed consent.

Sampling

Nasal biopsy samples and PBMCs were obtained from all subjects. The aim was to sample patients with WRA within 24 hours after relevant exposure. None of the study participants had respiratory infection within 2 weeks before sampling, and they were advised to stop using nasal corticosteroids 1 month earlier when possible.

ICS course for healthy controls

For safety reasons, inhaled corticosteroids (ICSs) were not withdrawn from the asthma patients before sample collection. To control the systemic effect of ICSs in the analysis, we assessed the effect ICS use in the healthy controls, who used a high-dose ICS (budesonide administered via a dry powder inhaler at a dose of 800 μg per day) for 5 to 6 weeks; PBMC samples were taken before and after the treatment.

Fig E1 (available in this article's Online Repository at www.jacionline.org) 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 Methods section of the Online Repository (available at www.jacionline.org).

Data analysis

SPSS, version 25.0, software (IBM Corporation, Armonk, NY) was used for analysis of demographic and clinical parameters. Medians and interquartile ranges were used to summarize continuous measures, whereas categoric variables were defined by their frequencies and proportions. Between-group differences were analyzed by using the Kruskal-Wallis test for continuous variables and chi-square tests for categoric variables. The eUTOPIA bioinformatic package[19] was used for transcriptomics analysis. After probe filtering and quantile normalization, batch effects originating from labeling and array variance were removed (the SVA package with Combat function).[20] Differential analysis was performed by Limma model analysis[21] by using age, body mass index (BMI), smoking status, and ICS use as covariates where applicable. A fold change of 1.5 or greater and a Benjamini-Hochberg adjusted *P* value less than .05 were considered indicative of significantly differentially expressed genes (DEGs).

Clusters and heatmaps were generated by using the Perseus program.[22] K-means clustering and principal component analysis (PCA) were used to visualize clustering of the gene profiles. The physiologic implications of the DEGs were predicted via pathway enrichment analyses by using Ingenuity Pathway Analysis tool (QIAGEN, Hilden, Germany).

RESULTS

Demographic and clinical characteristics

The study population comprised 61 men: 46 patients with WRA who were exposed to flours (the FA group; n = 15), isocyanates (the IA group; n = 20), or welding fumes (the WA group; n = 11) and 15 heathy control subjects. In the IA group, 11 subjects were exposed to methylene diphenyl diisocyanate, 7 were exposed to hexamethylene diisocyanate, and 4 were exposed to toluene diisocyanate. In the WA group, all participants were exposed to stainless steel fumes in their workplace. A total of 15 participants were diagnosed with occupational asthma. We did not detect statistically significant differences between the study groups in terms of age, smoking status, BMI, or atopy (Table I[23]). Duration and frequency of work exposure, symptomatic work exposure, and 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 ($P < .001$). Blood eosinophil count did not differ significantly between the study groups. Total IgE level ($P = .004$) and fractional exhaled nitric oxide (FENO) level ($P = .011$) were highest in the FA group. During sampling, 30 patients with asthma (65%) used an ICS. Lung function did not differ significantly between the groups, and the patients with asthma had more bronchial hyperresponsiveness than the controls did ($P = .004$). 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 patients with WRA (85%) (Table I and see Table E1 [available in this article's Online Repository at www.jacionline.org]).

Gene expression is significantly stronger, especially in nasal biopsy samples, in WA patients than in FA and IA patients

Microarray-based transcriptomic profiling was carried out on RNA extracted from both nasal biopsy samples and PBMCs. The

TABLE I. Characteristics of the 61 men participating in the study

Characteristic	FA (n = 15)	IA (n = 20)	WA (n = 11)	Healthy controls (n = 15)	*P* value
Age (y), median (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)	.553
Current smoking, no. (%)	1 (7)	2 (10)	2 (18)	2 (13)	.820
BMI, kg/m^2, median (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)	.074
Atopy, no. (%)*	10 (67)	10 (50)	7 (64)	4 (27)	.125
Duration of work exposure (y), median (IQR)	10.1 (6.8-30.0)	9.8 (1.9-19.4)	12.3 (8.0-20.0)	NA	.531
Frequency of work exposure, no. (%)					.314
Daily ≥1 h	13 (87)	11 (55)	9 (82)	NA	
Daily <1 h	1 (7)	2 (10)	1 (9)	NA	
≥1/wk	1 (7)	5 (25)	0 (0)	NA	
<1/wk	0 (0)	2 (10)	1 (9)	NA	
Duration of asthma symptoms at work (y), median (IQR)	2.0 (1.0-5.0)	1.0 (0.4-4.0)	4.0 (1.75-5.0)	NA	.138
Asthma control test score, median (IQR) (n = 42)	21 (16-24)	18 (15-23)	20 (17-23)	NA	.560
Use of ICS within a month, no. (%)	7 (47)	14 (70)	9 (82)	0 (0)	<.001
ICS dose, no. (%) (n = 30)†					.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, no. (%) (n = 60)	12 (80)	12 (63)	8 (73)	0 (0)	<.001
Nasal corticosteroid within 1 mo	2 (13)	3 (15)	1 (9)	0 (0)	.484
Total IgE level, kU/L, median (IQR)	101 (63-498)	57 (24-141)	79 (23-207)	23 (6-34)	.004
Blood eosinophils/μL, median (IQR) (n = 60)	210 (140-360)	220 (143-293)	140 (108-278)	160 (70-330)	.538
FENO level, median (IQR)	39 (24-63)	19 (12-28)	18 (14-32)	15 (8-24)	.011
FEV_1 % pred, median (IQR)	80 (71-87)	87 (77-93)	91 (76-97)	86 (75-95)	.160
FEV_1/FVC, median (IQR)	75 (66-79)	75 (72-77)	76 (70-78)	79 (74-81)	.250
Nonspecific bronchial hyperresponsiveness, no. (%),					.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‡	15 (100)	16 (80)	8 (73)	NA	.117

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.
FVC, Forced vital capacity; *IQR*, interquartile range; *NA*, not available; *PEF*, peak expiratory flow; *SPT*, skin prick test.
*Positive SPT to at least 1 environmental allergen.
†According to the Global Initiative for Asthma guidelines.[23]
‡Specific IgE or specific inhalation challenge to occupational agent positive, or workplace PEF suggestive of WRA.

top 2 principal components, explaining 45% (in the case of nasal biopsy samples) and 59% (in the case of PBMCs) of the variation between the samples, are shown in Fig E2 (available in this article's Online Repository at www.jacionline.org). In the nasal biopsy samples, only individuals with WA clustered separately from the controls (Fig E2, *A*). All 3 WRA categories were visibly distinct from the controls in the PBMC samples (Fig E2, *B*). To identify disease-relevant genes for each exposure group, the nasal biopsy sample and blood cell gene expression profiles of the control subjects were compared with those of each of the 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 the WA patients (Fig 1, *A*). In the nasal biopsy samples, only 1 DEG was identified in the FA group, 24 DEGs were identified in the IA group, and 664 DEGs were identified in the WA group compared with in the controls (Fig 1, *B*). In their PBMCs, the FA patients exhibited 692 DEGs, the IA patients exhibited 585 DEGs, and the WA group exhibited 1544 DEGs compared with the controls (Fig 1, *C*). Taken together, these data indicate that much larger numbers of DEGs were detected in the PBMCs from each WRA exposure group than in their nasal biopsy samples.

Disease-related immune functions are suppressed in the PBMCs from all asthma exposure groups but enhanced in the nasal biopsy samples from WA patients

To gain further insight into the underlying mechanisms of disease, all of the 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 (Fig 2, *A* and *B*), the most significant gene functions were shared by the members of the IA and WA groups (nasal biopsy DEGs) and between the FA, IA and WA groups (PBMC DEGs). In the nasal biopsy samples, the top significant functions common to the IA and WA categories were *quantity of antigen presenting cells* and *quantity of leukocytes* (Fig 2, *A*). In the 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 (Fig 2, *B*). A heatmap based on significantly enriched pathways separates the nasal biopsy sample groups from the blood cell sample groups and further substantiates WA as the most pathologic asthma group in this cohort (Fig 2, *C*). Similar to the gene expression profiles, the significance

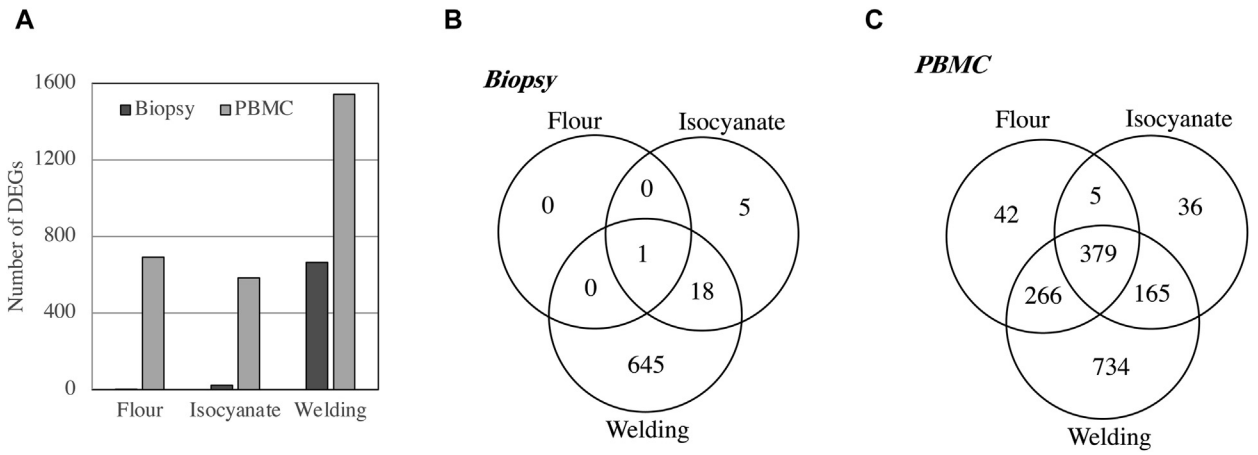

FIG 1. Overview and comparison of disease-relevant genes identified from nasal biopsy sample and blood cell transcriptome profiles of patients with asthma related to flour, isocyanates, and welding fumes. The highest numbers of DEGs were identified in patients whose asthma was related to welding fumes (**A**). With only 1 and 24 DEGs, respectively, the nasal biopsy transcriptomes of patients with FA and IA were quite similar to those of the controls. The Venn diagrams of the DEGs identified in nasal biopsy samples (**B**) and PBMCs (**C**) suggest overlapping disease mechanisms across the different types of asthma in this cohort.

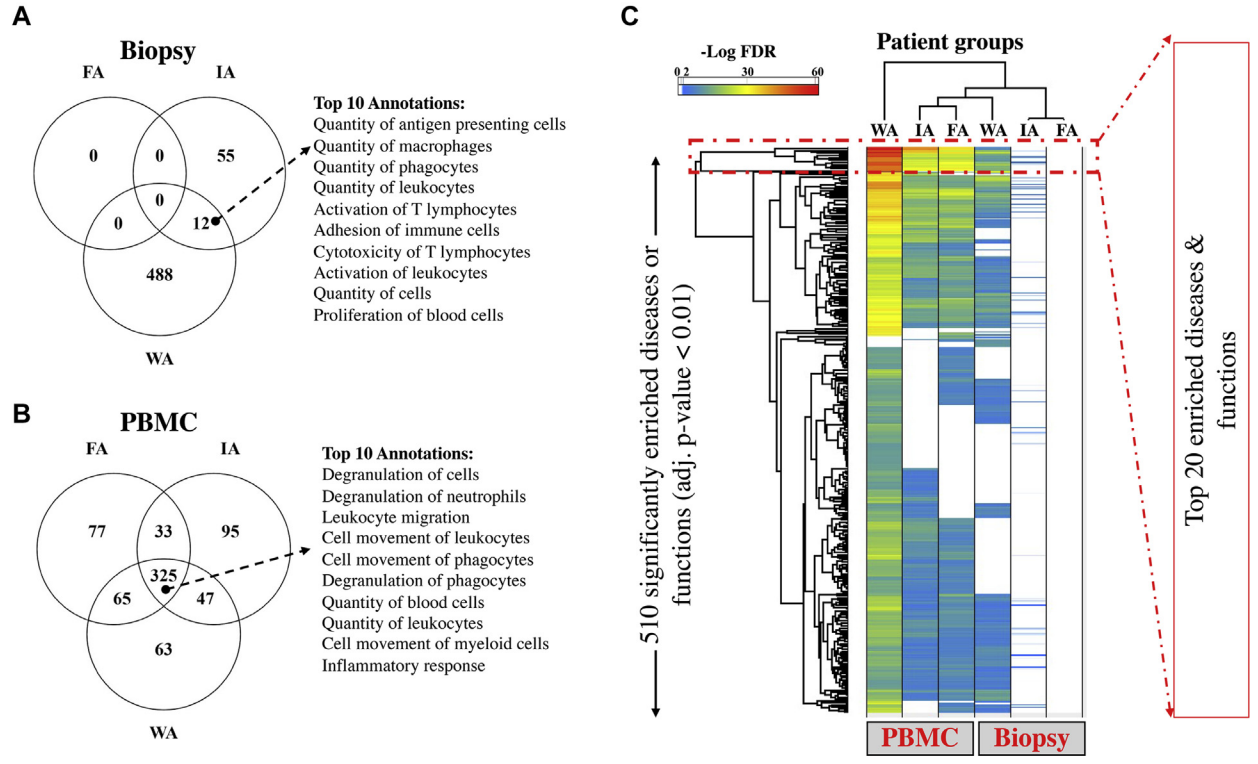

FIG 2. Gene function enrichment analyses. Venn comparisons of significantly overrepresented disease and biofunctions within DEGs identified in nasal biopsy samples (**A**) and PBMCs (**B**). Overlapping pathways in both tissue types correspond to the most significantly enriched gene functions. **C,** A hierarchic cluster of all enriched pathways based on the negative log of the pathway false discovery rate (FDR) (negative log of the FDR) suggests greater similarity of disease mechanisms between FA and IA. Nonenriched pathways are shown in white, whereas the color gradient from blue through yellow to red depicts increasing significance of the identified enriched disease and biofunction pathways.

of enriched disease or biofunctions was greater in the PBMCs than in the nasal biopsy samples (Fig 2, *C*).

Ranking of the top 20 enriched functions and a hierarchic cluster of their predicted activation status are shown in Fig 3, *A* and *B,* respectively. Genes involved in *leukocyte migration*, *degranulation of neutrophils*, and general *inflammatory response* were highly represented (a false discovery rate of 1E–58.1 to 1E–15.6) in the DEGs from the peripheral blood

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

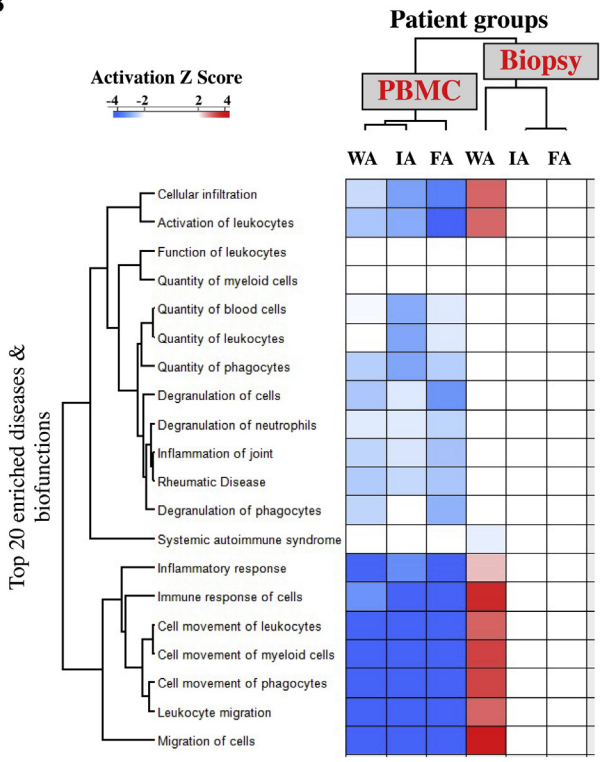

FIG 3. Comparative ranking (negative log of the false discovery rate [FDR]) of the topmost pathways enriched by DEGs across each asthma exposure group (**A**). Activity of each pathway was predicted by using the activation *z* score. A heatmap of these pathways, based on their activation states, is shown in (**B**). Only heat signatures of pathways with activation *z* scores exceeding the significance threshold of no more than –2 for suppression and at least 2 for activation are shown in the heatmap. All functions that are suppressed in the PBMC gene profile are predicted to be activated in the nasal biopsy sample gene profile.

cells of the FA, IA, and WA groups (Fig 3, *A*). The DEGs in the nasal biopsy samples of the WA patients represent the same diseases and biofunctions as the DEGs identified in the blood cells of the FA, IA, and WA patients. However, the underlying pathways are predicted to be activated in the nasal biopsy sample gene profiles of the FA, IA, and WA patients, the same pathways are predicted to be inactivated in the PBMCs of WA patients (Fig 3, *B*).

We next sought to answer the question of whether some of these DEGs are under the control of the upstream inflammatory regulators that play important roles in immune system disorders. The top 20 upstream regulators predicted in the Ingenuity Pathway Analysis are shown in Fig E3, *A* (available in this article's Online Repository at www.jacionline.org). These regulators include the proinflammatory cytokines TNF, IFN-γ, TGF-β1, IL-1β, and CSF2, as well as anti-inflammatory cytokines such as IL-13, IL-4, IL-6, and IL-10. Yet again, the nasal biopsy sample gene expression profile was consistent with activation of these cytokines, the PBMC gene expression profile revealed suppression or inactivation of these same cytokines (see Fig E3, *B*). The trend in expression of selected upstream regulators is in line with the activation states predicted from the expression profiles of their downstream targets (see Fig E4 [available in this article's Online Repository at www.jacionline.org]).

Gene-phenotype correlations reveal genes with high clinical relevance

Hierarchic clustering based on the Pearson correlation coefficient revealed 2 distinct clusters of clinically relevant disease features (see Fig E5 [available in this article's Online Repository at www.jacionline.org]). Clinical measures of lung function, eosinophilic inflammation, bronchial hyperresponsiveness, atopy, and asthma control make up cluster 1, whereas 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 (see Fig E5).

We next sought to answer the question of 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 3 asthma groups, genes with a Pearson correlation coefficient (R) of –0.6 to 0.6 to at least 1 clinical feature were included. A heatmap illustrating correlations of DEGs to diagnostically relevant clinical criteria in each of the patient groups is shown in Fig 4. The correlated genes and their corresponding correlation coefficients are provided in Table E2 (available in this article's Online Repository at www.jacionline.org). PCA analysis showed that the expression profile of these highly correlating genes clustered the patients separately from the controls in all of the asthma groups (Fig 4, *A-C* [*lower panel*]). Bronchial hyperresponsiveness had the strongest

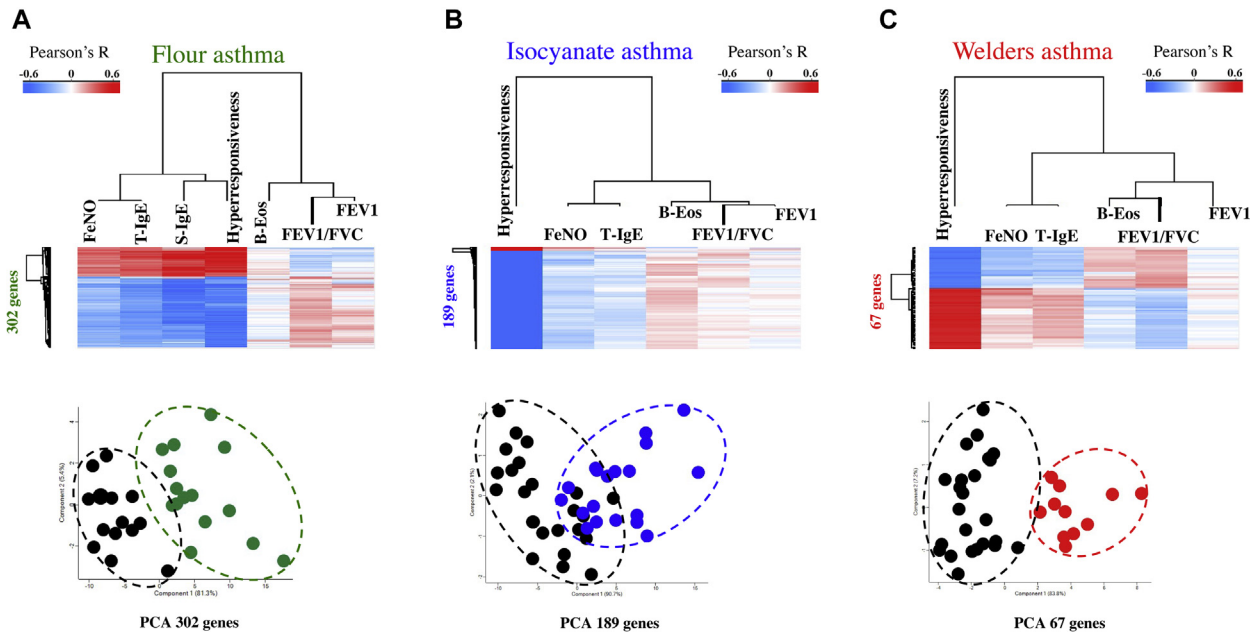

FIG 4. Heatmaps of the correlation (Pearson) between DEGs identified in blood cells of each asthma exposure group and selected clinical features with relevance for asthma, in the FA/control (**A** [*upper panel*]), IA/control (**B** [*upper panel*]), and WA/control (**C** [*upper panel*]) groups. The clinical features correlated with the identified DEGs are FENO level, specific IgE (S-IgE) level, total IgE (T-IgE) level, airway hyperresponsiveness (Hyperresponsiveness), blood eosinophil (B-Eos) count, and lung function (FEV_1-to-forced vital capacity [FVC] ratio and FEV_1%). PCA plots based on genes with a correlation (*R*) of –0.6 to >0.6 to at least 1 clinical parameter are shown for each asthma group (**A-C** [*lower panel*]).

correlation to gene expression for all of the asthma groups (Fig 4, *A-C*). The gene correlations with all other clinical parameters were weak or nonexistent, except for specific IgE level and total IgE level, which had the strongest correlation with gene expression in the FA group (Fig 4, *A*).

Similarly, we identified several DEGs in the PBMCs that were associated ($–0.6 \leq R \geq 0.6$) with at least 1 parameter from cluster 2. A heatmap of the correlated genes for each WRA exposure group is depicted in Fig E6, *A* (available in this article's Online Repository at www.jacionline.org). The strongest modulators of gene expression among the WRA patients were ICS use in all WRA subtypes; exposure frequency in the IA subtype; smoking in the IA and WA subtypes; and age, exposure duration, symptom duration, and BMI in the FA subtype. The top enriched biologic processes represented by patient demographics–associated DEGs in all WRA subtypes was *regulation of cytokine production* (see Fig E6, *B*).

Biomarker sets with only 5 genes accurately identify WRA exposure–related endotypes

We next sought to identify endotype biomarkers that could facilitate diagnosis by identification of group-specific DEGs that are associated with clinical features. Fig 4 shows that 302, 189, and 67 genes were associated with at least 1 clinical feature in the FA, IA, and WA patients, respectively. A Venn comparison of these clinical feature–associated DEGs is shown in Fig E7, *A* (available in this article's Online Repository at www.jacionline.org), and the most significant pathways represented by these genes are shown in Fig E7, *B*. The 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 DEGs that are associated with diagnostically relevant clinical features. This way we end up with genes that are both associated with at least 1 clinical feature and uniquely differentially expressed in that particular asthma group. The unique DEGs (Fig 1, *C*) comprised 42, 36, and 734 genes in the FA, IA, and WA endotypes, respectively. The expression profile of these asthma exposure group–specific DEGs in PBMCs separated all of the patients from the controls (see Fig E7, *C*). The separation of patient samples 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 (see Fig E7, *D*). We then calculated the intraclass correlation coefficients for each of these genes (see Table E3 in this article's Online Repository at www.jacionline.org).

The PCA plots based on the expression profile of the top 5 genes for each of the asthma exposure groups are shown in Fig 5. We propose these top 5 genes as biomarker panel candidates for FA (Fig 5, *A*), IA (Fig 5, *B*), and WA (Fig 5, *C*). These biomarkers are as follows: downregulation of *MYOM2*, *CDK5R1*, *ZDBF2*, and *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

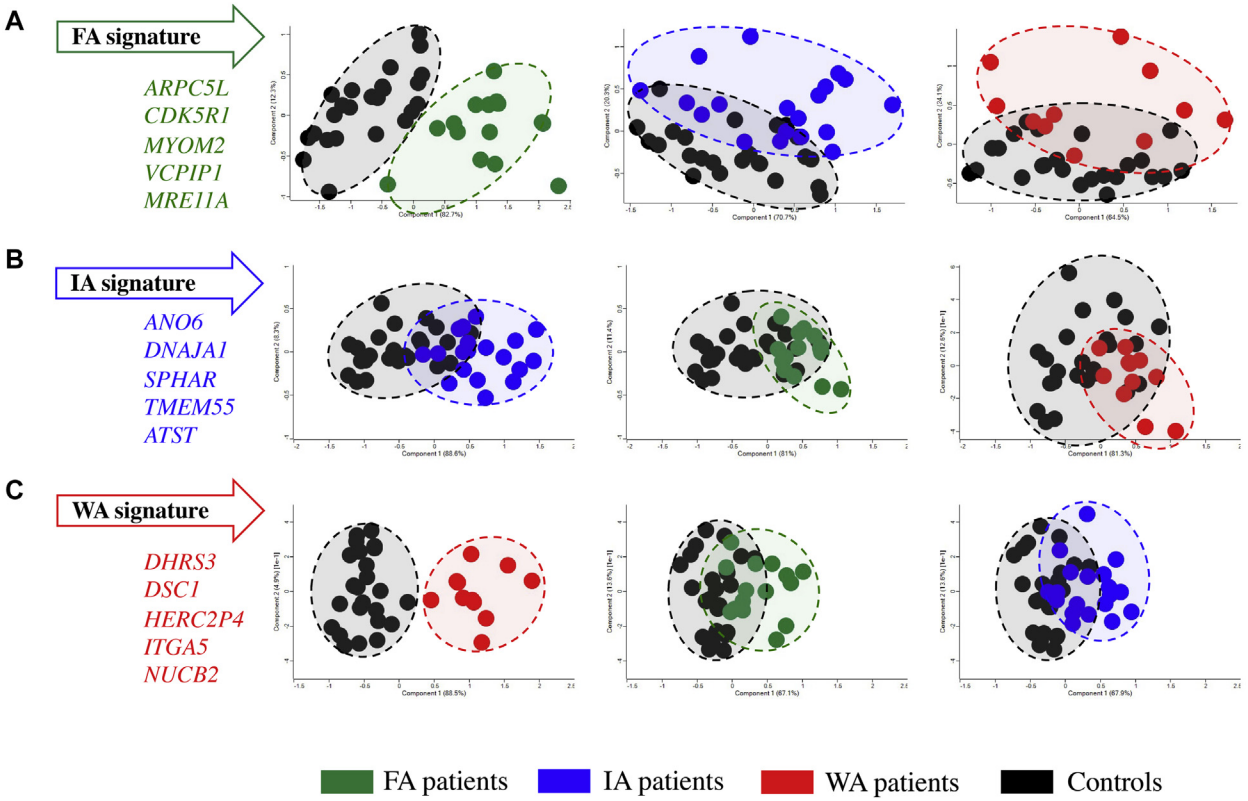

FIG 5. Exposure group–specific biomarkers were identified as subsets of genes that are differentially expressed in only 1 asthma group and whose expression profile is correlated ($-0.6 < R > 0.6$) with at least 1 clinical feature. A PCA plot depicting asthma group separation from the controls based on the top 5 biomarker candidate DEGs specific to FA (**A**), IA (**B**), and WA (**C**) is shown. From left to right, the best distinction between patients and controls is always observed for the asthma group from which the potential biomarker genes were identified as uniquely differentially expressed and correlated with a clinical diagnostic feature.

combination of clinical tests and transcriptomic analysis of blood immune cells and airway epithelium to investigate underlying disease mechanisms and identify endotype-specific biomarkers. Elaborating on pathologic mechanisms and identifying disease-associated biomarkers in WRA related to different exposures have the potential to improve diagnosis and facilitate the development of targeted treatment.

We studied WRA patients with exposure to 3 major workplace agents (ie, flour, isocyanates, and welding fumes) associated with different disease mechanisms. 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 a tertiary occupational medicine clinic in which the patient population was preselected to have a high probability of occupational disease. The 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 at detection of sensitizer-induced asthma, whereas welding fumes act via different mechanisms.[11] These tests may select cases on the basis of disease mechanisms, may give false-negative results, and cannot reliably distinguish occupational asthma from work-exacerbated asthma.[6,7] We studied WRA without preselection based on clinical testing and used the same inclusion criteria in all exposure groups (asthma emergence and aggravation of symptoms following exposure to specific occupational agents). Nevertheless, we detected biomarkers that identify each WRA exposure group, supporting homogeneity and similar background mechanisms among the participants in each group. Overall, our results shed light on mechanisms of asthma related to certain work exposures rather than on mechanisms of WRA categories (occupational or work-exacerbated asthma), and the results cannot be applied in the differential diagnosis of them. All of the WRA patients fulfilled the diagnostic criteria of asthma before sampling. At the time of sampling, most of the patients were using an ICS, and their clinical test results showed mainly well or partly controlled asthma.

To diminish heterogeneity of the study population, all of the participants were male and all of the WRA patients were currently exposed to the relevant occupational agent. Demographic variables did not differ between the groups. Because ICS treatment had a great effect on transcriptome in previous studies,[24] to avoid bias related to ICS, steroid effect was examined in healthy controls and taken into account in the analysis. Similar to in previous studies, most of the WRA patients reported work-related rhinitis.[25,26] Rhinitis is considered a manifestation of united

airway disease including both upper and lower airways rather than being a separate phenomenon.[27] Similar to in previous studies, asthma related to high-molecular-weight allergens (flour) showed IgE-mediated sensitization to the occupational agent and had a high FENO level reflecting T2-derived inflammation[25,28] and representing the T2 phenotype of asthma; in contrast, the IA group was largely not IgE-associated, and T2 inflammation was not detected in the IA or WA groups.

We selected nasal epithelium as a local sampling site because in previous studies the quality of nasal epithelial samples has been sufficient for transcriptomic analysis,[29] whereas the quality of sputum samples has varied.[30] Localized (nasal biopsy samples) and systemic (PBMCs) gene expression was profiled after exposure to either flour, isocyanates, or welding fumes. The several times higher number of DEGs in blood immune cells relative to the number in nasal biopsy samples suggests that the pathomolecular changes existed predominantly in the systemic circulation at the time of sampling. Our results also suggest that ongoing inflammation in the airway epithelium in patients with FA or IA was virtually nonexistent. It is possible, however, that the use of ICSs may partially explain why inflammation in the airway epithelium could not be identified in these WRA groups. In contrast, exposure to welding fumes was related to changes in gene expression that were detectable both in the airway epithelium and in PBMCs, which is indicative of a hazardous inflammatory process in the airways. On the basis of an extensive literature search, it appears that comparable comprehensive transcriptomics studies in human subjects are practically nonexistemt,[31-34] and to our knowledge, there are no comparable studies of WRA. A few well-designed studies have been reported in mice[35-38] and rats,[39-41] but their relevance in the identification of disease endotype–specific biomarkers in humans is questionable.[42]

With regard to the most robust results derived from the pathway enrichment analysis, all of the asthma groups exhibited significant suppression of immune functions related to cell migration and cell movement in blood immune cells. Only WA patients demonstrated enrichment of cell migration–related immune functions, but contradictory to what was observed in PBMCs, those pathways were enhanced in the airway epithelia. Similar phenomena could also be seen with predicted upstream regulators in WA patients: several inflammatory cytokines (eg, TNF, IFN-γ, IL-1β, and TGF-β1) were suppressed or inactivated in the blood immune cells but stimulated or activated in the nasal epithelia. This suggests that the changes in gene expression observed in WA patients, both in their nasal epithelia and in their peripheral blood cells, are controlled by a central pathologic mechanism. It can be speculated that these contradictory gene expression profiles are due to differences in the inflammatory cell pools in the nasal epithelium and in the circulation. In other words, the inflammatory cells that are likely the main source of the exposure-related gene expression are recruited to the site of the inflammation (ie, the airway epithelia) and are therefore missing from the circulation. In the nasal biopsy samples, because of the limited number of identified DEGs, it is unsurprising that very few to no significantly enriched immune functions were identified from the FA and IA 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 with gene expression in all of the asthma groups. Interestingly, FENO level and specific and total IgE levels demonstrated moderate correlations with gene expression in the FA group but not in the other groups. This is likely due to the more prominent IgE-associated and T2-type immunity in the FA patients than in the IA and WA 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 effects of smoking status, subject age, BMI, and ICS dose were adjusted during between-group (patient vs control) differential expression analysis. Within each WRA exposure group, however, in addition to exposure duration and frequency and symptom duration, these factors 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 proinflammatory and/or anti-inflammatory signaling. This finding is consistent with the findings of a previous report indicating that the severity of asthma is associated with older age, higher BMI, and higher rate of tobacco smoking[43] and with a report of an association of severity of occupational asthma with persistent exposure to the relevant occupational agent and longer duration of symptomatic exposure.[44] 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, which is indicative of similarities in the pathologic mechanisms, exposure-specific differences were also found. To identify specific exposure-related biomarkers, we combined and analyzed the information on the unique DEGs identified in PBMCs, as well as information on the DEGs that are associated with the clinical parameters. Our prioritization analysis revealed specific sets of 5 candidate genes for each exposure-related endotype that are 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 sheds light on asthma mechanisms related to 3 different types of exposures present at workplace. Active inflammation in airway epithelia could be detected from the gene expression only in WA. In contrast, large numbers of DEGs were detected in the blood immune cells in all of the patient groups. 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 of accurately identifying asthma related to flour, isocyanates, or welding. Our results could provide a basis for future attempts to create diagnostic biomarkers for clinical settings.

We thank Sirpa Hyttinen, Tanja Katovich, and the study nurses for their assistance in this study, as well as Terhi Vesa, MSc, Varpu Alenius, and Mikko Asiala for their excellent help in processing cell samples. Additionally, we thank Professor Dario Greco for valuable discussions on the study design.

Key messages

- **Many DEGs were detected in blood immune cells of patients with work-related asthma. 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 the study patients.**
- **In transcriptome-phenotype correlations, hyperresponsiveness had the highest clinical relevance and was associated with a set of genes specific to asthma related to flour, isocyanate, or welding fume exposure.**
- **Biomarker sets containing 5 genes capable of accurate identification of the endotypes have been identified for each of the work-related asthma exposure groups.**

REFERENCES

1. Kaur R, Chupp G. Phenotypes and endotypes of adult asthma: moving toward precision medicine. J Allergy Clin Immunol 2019;144:1-12.
2. Blanc PD, Annesi-Maesano I, Balmes JR, Cummings KJ, Fishwick D, Miedinger D, et al. The occupational burden of nonmalignant respiratory diseases. An official American Thoracic Society and European Respiratory Society atatement. Am J Respir Crit Care Med 2019;199:1312-34.
3. Maestrelli P, Henneberger PK, Tarlo S, Mason P, Boschetto P. Causes and phenotypes of work-related asthma. Int J Environ Res Public Health 2020;17:4713.
4. Lau A, Tarlo SM. Update on the management of occupational asthma and work-exacerbated asthma. Allergy Asthma Immunol Res 2019;11:188-200.
5. Tarlo SM, Quirce S. Impact of identification of clinical phenotypes in occupational asthma. J Allergy Clin Immunol Pract 2020;8:3277-82.
6. Chiry S, Cartier A, Malo JL, Tarlo SM, Lemière C. Comparison of peak expiratory flow variability between workers with work-exacerbated asthma and occupational asthma. Chest 2007;132:483-8.
7. Vandenplas O, Suojalehto H, Aasen TB, Baur X, Burge PS, de Blay F, et al. Specific inhalation challenge in the diagnosis of occupational asthma: consensus statement. Eur Respir J 2014;43:1573-87.
8. Lummus ZL, Wisnewski AV, Bernstein DI. Pathogenesis and disease mechanisms of occupational asthma. Immunol Allergy Clin North Am 2011;31:699-716, vi.
9. Bernstein DI, Lummus ZL, Kesavalu B, Yao J, Kottyan L, Miller D, et al. Genetic variants with gene regulatory effects are associated with diisocyanate-induced asthma. J Allergy Clin Immunol 2018;142:959-69.
10. Choi Y, Sim S, Park HS. Is TLR4 Critical for neutrophil apoptosis in occupational asthma? Allergy Asthma Immunol Res 2020;12:560-2.
11. Riccelli MG, Goldoni M, Poli D, Mozzoni P, Cavallo D, Corradi M. Welding fumes, a risk factor for lung diseases [abstract]. Int J Environ Res Public Health 2020;17:2552.
12. Banga A, Reilly MJ, Rosenman KD. A study of characteristics of Michigan workers with work-related asthma exposed to welding. J Occup Environ Med 2011;53:415-9.
13. Hannu T, Piipari R, Tuppurainen M, Nordman H, Tuomi T. Occupational asthma caused by stainless steel welding fumes: a clinical study. Eur Respir J 2007;29:85-90.
14. Wurtz ET, Schlunssen V, Malling TH, Hansen JG, Omland O. Occupational COPD among Danish never-smokers: a population-based study. Occup Environ Med 2015;72:456-9.
15. Giovannini-Chami L, Marcet B, Moreilhon C, Chevalier B, Illie MI, Lebrigand K, et al. Distinct epithelial gene expression phenotypes in childhood respiratory allergy. Eur Respir J 2012;39:1197-205.
16. Bigler J, Boedigheimer M, Schofield JPR, Skipp PJ, Corfield J, Rowe A, et al. A severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts. Am J Respir Crit Care Med 2017;195:1311-20.
17. Jiang Y, Gruzieva O, Wang T, Forno E, Boutaoui N, Sun T, et al. Transcriptomics of atopy and atopic asthma in white blood cells from children and adolescents. Eur Respir J 2019;53:1900102.
18. Raedler D, Ballenberger N, Klucker E, Bock A, Otto R, Prazeres da Costa O, et al. Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. J Allergy Clin Immunol 2015;135:81-91.
19. Marwah VS, Scala G, Kinaret PAS, Serra A, Alenius H, Fortino V, et al. eUTOPIA: solUTion for Omics data preprocessing and analysis. Source Code Biol Med 2019;14:1.
20. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinformatics 2012;28:882-3.
21. G.K. S. limma: Linear Models for microarray data. In: Gentleman R, Carey VJ, Huber W, Irazarry RA, Dudolt S, editors. Bioinformatics and computational biology solutions using R and bioconductor statistics for biology and health. New York, NY: Springer; 2005.
22. Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, et al. The Perseus computational platform for comprehensive analysis of (prote)omics data. Nat Methods 2016;13:731-40.
23. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention 2020. Available at: http://www.ginasthma.org/. Accessed March 5, 2021.
24. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci U S A 2007;104:15858-63.
25. Vandenplas O, Godet J, Hurdubaea L, Rifflart C, Suojalehto H, Wiszniewska M, et al. Are high- and low-molecular-weight sensitizing agents associated with different clinical phenotypes of occupational asthma? Allergy 2019;74:261-72.
26. Vandenplas O, Van Brussel P, D'Alpaos V, Wattiez M, Jamart J, Thimpont J. Rhinitis in subjects with work-exacerbated asthma. Respir Med 2010;104:497-503.
27. Castano R, Gautrin D, Theriault G, Trudeau C, Ghezzo H, Malo JL. Occupational rhinitis in workers investigated for occupational asthma. Thorax 2009;64:50-4.
28. Lemiere C, NG S, Sava F, D'Alpaos V, Huaux F, Vandenplas O. Occupational asthma phenotypes identified by increased fractional exhaled nitric oxide after exposure to causal agents. J Allergy Clin Immunol 2014;134:1063-7.
29. Drizik E, Corbett S, Zheng Y, Vermeulen R, Dai Y, Hu W, et al. Transcriptomic changes in the nasal epithelium associated with diesel engine exhaust exposure. Environ Int 2020;137:105506.
30. Matsuoka H, Niimi A, Matsumoto H, Ueda T, Takemura M, Yamaguchi M, et al. Patients' characteristics associated with unsuccessful sputum induction in asthma. J Allergy Clin Immunol 2008;121:774-6.
31. Gomez-Casado C, Garrido-Arandia M, Pereira C, Catarino M, Parro V, Armentia A, et al. Component-resolved diagnosis of wheat flour allergy in baker's asthma. J Allergy Clin Immunol 2014;134:480-3.
32. Rim KT, Park KK, Kim YH, Lee YH, Han JH, Chung YH, et al. Gene-expression profiling of human mononuclear cells from welders using cDNA microarray. J Toxicol Environ Health A 2007;70:1264-77.
33. Taylor LW, French JE, Robbins ZG, Boyer JC, Nylander-French LA. Influence of genetic variance on biomarker levels after occupational exposure to 1,6-hexamethylene diisocyanate monomer and 1,6-hexamethylene diisocyanate isocyanurate. Front Genet 2020;11:836.
34. Wang Z, Neuberg D, Su L, Kim JY, Chen JC, Christiani DC. Prospective study of metal fume-induced responses of global gene expression profiling in whole blood. Inhal Toxicol 2008;20:1233-44.
35. Erdely A, Antonini JM, Salmen-Muniz R, Liston A, Hulderman T, Simeonova PP, et al. Type I interferon and pattern recognition receptor signaling following particulate matter inhalation. Part Fibre Toxicol 2012;9:25.
36. Lee CT, Ylostalo J, Friedman M, Hoyle GW. Gene expression profiling in mouse lung following polymeric hexamethylene diisocyanate exposure. Toxicol Appl Pharmacol 2005;205:53-64.
37. Wisnewski AV, Liu J, Redlich CA. Analysis of lung gene expression reveals a role for Cl(-) channels in diisocyanate-induced airway eosinophilia in a mouse model of asthma pathology. Am J Respir Cell Mol Biol 2020;63:25-35.
38. Zeidler-Erdely PC, Kashon ML, Li S, Antonini JM. Response of the mouse lung transcriptome to welding fume: effects of stainless and mild steel fumes on lung gene expression in A/J and C57BL/6J mice. Respir Res 2010;11:70.
39. Erdely A, Antonini JM, Young SH, Kashon ML, Gu JK, Hulderman T, et al. Oxidative stress and reduced responsiveness of challenged circulating leukocytes following pulmonary instillation of metal-rich particulate matter in rats. Part Fibre Toxicol 2014;11:34.
40. Oh JH, Yang MJ, Heo JD, Yang YS, Park HJ, Park SM, et al. Inflammatory response in rat lungs with recurrent exposure to welding fumes: a transcriptomic approach. Toxicol Ind Health 2012;28:203-15.
41. Rim KT, Park KK, Sung JH, Chung YH, Han JH, Cho KS, et al. Gene-expression profiling using suppression-subtractive hybridization and cDNA microarray in rat mononuclear cells in response to welding-fume exposure. Toxicol Ind Health 2004;20:77-88.
42. Aun MV, Bonamichi-Santos R, Arantes-Costa FM, Kalil J, Giavina-Bianchi P. Animal models of asthma: utility and limitations. J Asthma Allergy 2017;10:293-301.
43. Mincheva R, Ekerljung L, Bossios A, Lundback B, Lotvall J. High prevalence of severe asthma in a large random population study. J Allergy Clin Immunol 2018;141:2256-64.e2.
44. Vandenplas O, Godet J, Hurdubaea L, Rifflart C, Suojalehto H, Walusiak-Skorupa J, et al. Severe occupational asthma: insights from a multicenter european cohort. J Allergy Clin Immunol Pract 2019;7:2309-18.e4.