Respiratory Susceptibility to Aerosolized Per- and Polyfluoroalkyl Substances in Human 3D Airway Models

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Abstract

Background and Purpose: Per- and polyfluoroalkyl substances (PFAS), including perfluorooctanoic acid (PFOA) and the replacement compound hexafluoropropylene oxide dimer acid (GenX, HFPO-DA), have emerged as chemicals of concern due to their potential associations with respiratory diseases. In this study, we conducted a systematic review to evaluate current studies of PFAS-related respiratory toxicity. We further investigated respiratory responses to PFAS by analyzing transcriptomic profiles using human 3D airway epithelial models.

Methods: In a systematic review following a PECO framework, we investigated 101 experimental studies covering epidemiology, in vivo and in vitro PFAS respiratory toxicity, and PFAS inhalation exposure. Normal and asthmatic MucilAir™ were exposed to aerosolized PFAS using an air-liquid interface Vitrocell® aerosol exposure system. Differentially expressed gene (DEG) profiling was performed, followed by gene ontology (GO), KEGG pathway, and cell type enrichment analyses based on single sample gene set enrichment analysis (ssGSEA). We identified responses related to allergic phenotypes and shifts in airway epithelial subtypes.

Results: First, limited articles were available for GenX. Meanwhile, PFOA has been extensively reported for associations with inflammation, impaired lung function, chronic obstructive pulmonary fibrosis (COPD), lung cancer, and airway allergies (wheezing and asthma) in human studies. Many in vivo and in vitro studies suggested fibrotic, tumor-associated, and asthmatic responses of PFOA. Following non-toxic aerosol exposure of PFOA and GenX to MucilAir™, GO and KEGG pathway analysis revealed T helper cell-related immune pathways across multiple comparisons of normal versus asthmatic tissues and PFAS exposures in asthmatic conditions. Notably, multiple major histocompatibility complex (MHC) class II genes were consistently upregulated with aerosolized PFAS exposure particularly in the asthmatic background. Consistent with these findings, ssGSEA results showed a shift in airway epithelial subtypes characterized by downregulated basal subtypes and upregulated secretory subtypes (secretory and goblet). These alterations were modest in normal tissues but markedly amplified in asthmatic airway models exposed to PFOA or GenX.

Conclusions: In summary, inhalation exposure of PFOA and GenX induce MHC class II phenotypes and shifts in airway epithelial subtypes. This indicates that aerosolized PFAS exposure may promote allergic epithelial states, particularly under asthmatic conditions.

Keywords: Per- and polyfluoroalkyl chemicals, Systematic review, Aerosol exposure (Vitrocell®), Transcriptomic analysis, Human 3D airway epithelial model

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Reference

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2. Deprez, M., Zaragosi, L. E., Truchi, M., Becavin, C., Ruiz García, S., Arguel, M. J., ... & Barbry, P. (2020). A single-cell atlas of the human healthy airways. *American journal of respiratory and critical care medicine*, *202*(12), 1636-1645.

Model preparation & aerosol exposure

Figure 1. MucilAir™ normal and asthma model

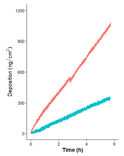

Figure 2. Aerosol generation of PFOA and GenX

Model preparation: Fully differentiated human bronchial epithelial tissues (MucilAir™, Epithelix, Switzerland) derived from normal and asthma donors were used as a human 3D airway epithelial model cultured under air–liquid interface conditions. Upon receipt, tissues were maintained according to the manufacturer's instructions at 37°C in a humidified atmosphere with 5% CO_2 and allowed to stabilize for 2 days prior to exposure.

Aerosol exposure: Aerosol exposure experiments were conducted on day 3 of stabilization. PFOA and GenX were prepared in sterile distilled water and aerosolized using a Vitrocell® (Waldkirch, Germany) exposure system designed for air–liquid interface cultures. The generated aerosol was delivered directly to the apical surface of the tissues under controlled airflow conditions. Deposited doses were quantified and expressed as ng/cm². Exposure durations were set at 30 min, 2 h, or 6 h depending on the experimental design, while control tissues were exposed to filtered air under 2 h exposure. Following exposure, tissues were incubated for post-exposure analyses including cytotoxicity assessment and transcriptomic profiling.

Table 1. Aerosol generation of PFOA

Parameter	Condition
Concentrate	0.35% PFOA with 0.1% DMSO in PBS
Flow meter	Total volume 110 mL
Injection speed	0.20 mL/min
WellFlow meter	3.5 mL/min
Aerosol generator	3.8-4.0 L/min
Exposure time	6h
Temperature	36.5°C

Table 2. Aerosol generation of GenX

Parameter	Condition
Concentrate	2.0% GenX with 0.1% DMSO in PBS
Flow meter	Total volume 110 mL
Injection speed	0.20 mL/min
WellFlow meter	3.5 mL/min
Aerosol generator	3.8-4.0 L/min
Exposure time	6h
Temperature	36.5°C

Result

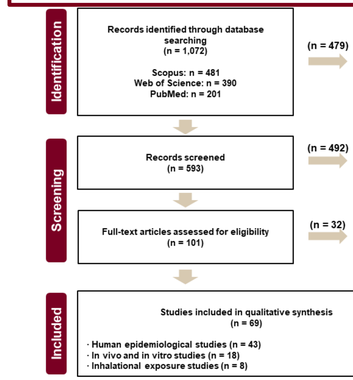

Figure 3. Systematic review for risk of PFOA and GenX in respiratory diseases

Method: A systematic literature search was conducted in Scopus, Web of Science, and PubMed to identify studies evaluating respiratory effects of PFOA and GenX. After duplicate removal and screening based on predefined inclusion criteria, 69 studies were included in the qualitative synthesis (43 epidemiological, 18 in vivo/in vitro, and 8 inhalation exposure studies).

Result: Epidemiological studies reported associations between PFAS exposure and asthma-related outcomes, including asthma prevalence, wheezing, and reduced lung function, although findings were heterogeneous across populations and PFAS congeners. In contrast, experimental studies consistently demonstrated immune modulation and asthmatic responses, supporting potential mechanistic links to allergic airway disease.

Result

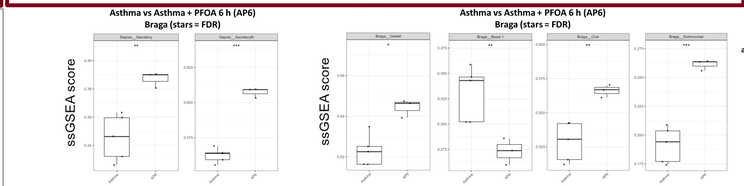

Figure 4. ssGSEA plots of PFOA-treated asthmatic MucilAir™ model

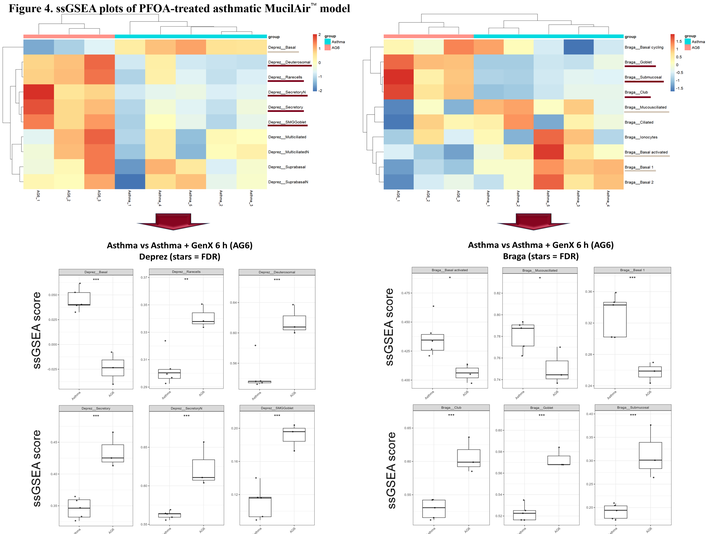

Figure 5. ssGSEA plots and heatmap of GenX-treated asthmatic MucilAir™ model

Method: RNA sequencing was performed to characterize transcriptional responses following PFAS exposure. RNA libraries were prepared using a TruSeq stranded mRNA library kit and sequenced on an Illumina platform. The raw reads were aligned to the human reference genome (GRCh38/hg38). Raw count data were processed using the edgeR package, where duplicated gene symbols were collapsed and lowly expressed genes were filtered. Library size normalization was performed using the trimmed mean of M-values (TMM) method, and normalized counts were transformed to log2 counts per million (log2CPM) values. Cell subtype enrichment was evaluated using ssGSEA implemented in the GSVA R package, using airway epithelial lineage signatures derived from Deprez et al. (2020) and asthma-associated epithelial signatures from Vieira Braga et al. (2019). Statistical differences in ssGSEA scores between experimental groups were assessed using linear modeling with empirical Bayes moderation (limma package). *p*-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) method. Significance levels are indicated as *FDR < 0.05, **FDR < 0.01, and ***FDR < 0.001.

Result: The analysis revealed a shift in epithelial cell subtype signatures following PFAS exposure. In both PFOA- and GenX-treated tissues, basal cell–associated gene signatures were reduced, whereas secretory epithelial signatures including secretory and goblet cell markers were relatively increased. These alterations were modest in normal tissues but more pronounced in the asthmatic airway model. Heatmap visualization further demonstrated that PFAS exposure was associated with coordinated changes across epithelial lineage-related gene sets, suggesting disruption of epithelial homeostasis and a shift toward secretory phenotypes commonly observed in allergic airway conditions.

Result

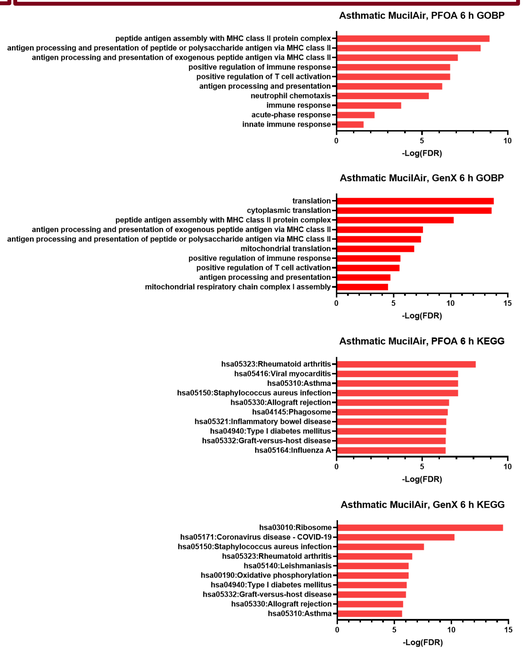

Figure 6. Enrichment analysis with GO BP and KEGG pathway

Method: Differentially expressed genes (DEGs) were identified using DESeq2, and functional enrichment analysis of DEGs was performed using DAVID (https://davidbioinformatics.nih.gov) to identify enriched gene ontology biological process (GO BP) and KEGG pathways. FDR: False Discovery Rate

Result: The enriched GO BP terms were predominantly associated with immune regulation and adaptive immune responses. Notably, pathways related to antigen processing and presentation, T helper cell activation, and immune response signaling were significantly enriched following PFAS exposure. Several MHC class II–related genes were consistently upregulated across chemical treatment, particularly in GenX. This may be due to relatively high concentration of GenX exposure than PFOA. These findings suggest that aerosolized PFAS exposure may promote immune activation and antigen presentation pathways within asthmatic airway epithelial tissues, which are known to contribute to allergic airway inflammation and asthma pathogenesis.

Conclusion

In summary, aerosolized exposure to PFOA and GenX induces immune-related transcriptional responses and shifts in airway epithelial subtypes in human airway models.

These findings suggest that PFAS inhalation exposure may promote allergic epithelial phenotypes, particularly in individuals with pre-existing airway diseases such as asthma.