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PFOA induces alteration in DNA methylation regulators and SARS-CoV-2 targets *Ace2* and *Tmprss2* in mouse lung tissues

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ABSTRACT

Perfluorooctanoic acid (PFOA), a ubiquitous environmental toxicant from the Per- and polyfluoroalkyl substances (PFAS) family has been implicated in toxicity of various organs. Several epidemiological studies have linked PFOA to different lung injuries and diseased conditions. However, the implication of PFOA in affecting epigenetic regulators and SARS-CoV-2 infection pathways in the lung are unknown. The present work explores the accumulation of PFOA in lungs and changes in mRNA expression of DNA methylation regulator genes DNA methyltransferases (*Dnmts*) and ten-eleven translocation (*Tets*) along with the membrane proteins angiotensin converting enzyme 2 (*Ace2*) and transmembrane Serine Protease 2 (*Tmprss2*) genes involved in the SARS-CoV-2 virus infection. CD1 mice were orally exposed to 5 and 20 mg/kg/day PFOA for 10 days and the lung tissues were analyzed using LCMS, qPCR, and pyrosequencing techniques. PFOA was shown to accumulate in the lung tissues and increase in a dose-dependent manner. *Dnmts* and *Tets* were significantly downregulated upon at least one of the PFOA dosing concentration, whereas *Ace2* and *Tmprss2* show significant increase in their expression level. Further, CpG islands in the promotor region of *Tmprss2* exhibited significant hypomethylation in PFOA treated groups, which supports its increased gene expression level. Current study reveals the implication of PFOA induced DNA methylation changes in lungs and their possible role in upregulation of *Ace2* and *Tmprss2*. It is possible that increased expression of these membrane receptors due to PFOA exposure can lead to higher susceptibility of SARS-CoV-2 infections.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals which contain more than 4000 compounds [1]. Perfluorooctanoic acid (PFOA), an 8-carbon PFAS has been widely used in household products and industrial applications since 1940s [2]. Due to their extensive use and high chemical stability, PFAS compounds are widespread in the environment [3]. PFOA has been detected in the blood serum of over 98 % of the US population [4,5]. Humans are exposed to PFOA through drinking water, agricultural products, inhalation of dust and medical materials and devices [6,7]. The long elimination half-life of PFOA which is ~2.4 years in humans [8], shows the low elimination rate from the body [9]. It is readily absorbed in the body and is not known to undergo metabolism or biotransformation in the cells [10]. The ubiquitous presence of PFOA in the environment and high bioaccumulation capability in body organs, make it a health concern for humans [4,11].

PFOA exposure has already been linked to the damage of different organs in several epidemiological, animal, and several *in vitro* studies. The primary toxicological effects observed in animal studies are liver, mammary, pancreatic, and testicular cancers [12], immune system suppression, obesity, and developmental toxicity [13]. Several epidemiological studies have linked PFOA to different type of cancers, ulcerative colitis, thyroid diseases [14], reduced response to vaccines [15], cardiovascular abnormalities [16], and decreased birth weight [17]. PFOA toxicity is well studied in the liver, kidney, and other body organs but very few experimental studies are reported on its toxicity in lungs. Lung is one of the primary exposed organs to this environmental contaminant by both inhalation [18] and oral ingestion [19]. Epidemiological studies link PFOA and other Per-fluorinated compounds to

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respiratory tract diseases, for example shortness of breath, asthma in adults [20], biomarkers of juvenile asthma in children [21], esophageal and lung cancer incidence in fire fighters [22].

Despite these studies, no reports are available to evaluate the effect of PFOA on epigenetic regulator enzymes and key lung membrane proteins (Ace2 and Tmprss2). We have already reported on the alteration of epigenetic regulators in the mice gut, liver, and kidneys, including *in vitro* studies with HepG2 and A549 due to PFOA exposure [23–26]. DNA methylation, one of the key epigenetic mechanisms is primarily regulated by DNMTs and TETs enzymes which further controls gene expression by adding or removing methyl groups on cytosine at specific Cytosine – Guanine (CpG) sites in those genes [27]. DNA methylation changes associated cellular mechanisms have been implicated with increased cancer risk among firefighters [28]. DNA methylation is altered in different pulmonary diseased conditions: cystic fibrosis [29], carcinoma [30], asthma [31], and recently in Covid-19 infections [32].

Angiotensin-converting enzyme2 (ACE2), one of the key regulators of the renin-angiotensin system (RAS) which functions as an enzyme and converts angiotensin-II to angiotensin (1–7) is found predominantly in the heart and kidney [33,34]. It also functions as a cell surface receptor in the respiratory tract to which different members of corona virus family such as SARS-CoV and the novel SARS-CoV-2 bind and gain entry into cells [35,36]. TMPRSS2, another membrane protein in the epithelial cells of lung, prostrate and gut tissues [37] is a serine protease which plays a role in proteolytic cascades for the normal physiological functions of tissue specifically prostrate. ACE2 has been frequently reported in several pathophysiological conditions, such as in cardiovascular, depression, pulmonary and renal damage [38], whereas TMPRSS2 is more often discussed in the context of prostate cancer [39]. These two key genes have been shown to play a major role in SARS-CoV-2 infection and are considered potential drug targets [40]. This viral infection leads to more fatalities in the individuals with higher expression level of ACE2 and TMPRSS2 receptors. A recent study linked blood serum level of the Perfluorobutanoic acid (PFBA) to Covid-19 hospitalization in these patients [41]. The epigenetic regulation of *ACE2* and *TMPRSS2* genes have long been reported and most recently in Covid-19 patients in the respiratory tract and lungs tissue [42,43].

We hypothesize that, PFOA can alter the expression of epigenetic regulators in the lungs to dysregulate the expression of *Ace2* and *Tmprss2* genes and affect the downstream associated pathways. We evaluated the accumulation of PFOA in mice lungs and monitored gene expression changes of the DNA methylating enzymes, Dnmts, and DNA demethylating enzymes Tets, and SARS-CoV-2 target genes *Ace2* and *Tmprss2*. Next, we analyzed the CpG methylation patterns of the promotor region of *Tmprss2* upon exposure to PFOA.

2. Methods

2.1. Chemicals and concentrations

PFOA (CAS# 335−67-1, 96 % purity) and Tween-20 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Two dosing concentrations 5 and 20 mg/kg/day of PFOA along with vehicle control were prepared in 0.5 % Tween-20 in deionized water. The concentrations were selected based on the reported amount of PFAS levels in the serum of people both from the environmental/community and occupational exposure. We decided our dosing concentrations (from low–high) and duration (10 days) taking into consideration the community and fluorochemical production workers exposure limits to PFOA [44–46].

2.2. Animals housing, dosing, and tissues collection

CD1 mice (an outbred strain) were used in this study to assess the possible effect of PFOA. Animal experiments were conducted with an approved protocol (Toxicology of Endocrine Disrupting Chemicals, Protocol# 19037) by the University of Illinois Urbana-Champaign, Institutional Animal Care and Use Committee (IACUC) per National Institute of Health (NIH) guidelines. Mice were acquired from Charles River, USA, and randomly divided into 3 groups (n = 5 mice per group) and housed in polysulfone, ventilated cages at room temperature on a 12:12 h light: dark cycle and given free access to the Teklad Rodent Diet 8604 and purified water.

PFOA dosing was initiated when mice were acclimatized to the new environment and reached the age of one month by oral gavage method. Two doses (5, and 20 mg/kg/day) of PFOA along with vehicle control were administered daily for 10 days. At the end of the dosing period, mice were euthanized with CO2 asphyxiation with a flow rate of 2.0 L/min to the mouse cage (8" x 13" x 5"). Lung tissues were collected immediately and placed in cryotubes after euthanasia, and stored in liquid nitrogen at the site of surgery and later transferred to the −80 ∘C freezer in the Lab.

2.3. Isolation of RNA and cDNA synthesis

Lung tissues were processed to extract total RNA by the Trizol method (Ambion, Thermofisher, Waltham, MA, USA) and dissolved in diethyl pyrocarbonate (DEPC) treated water (Invitrogen, Carlsbad, CA, USA). The concentration and purity of RNA were analyzed by NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Then, 1.5 μg of extracted RNA was reverse transcribed to cDNA from each sample using the High-Capacity cDNA synthesis kit (Applied Biosystems, Thermofisher, Waltham, MA, USA). The synthesized cDNA was diluted 25 times in molecular grade water (Corning Mediatech, Inc., Manassas, VA, USA). The final amount of cDNA used as a template was kept at 10 ng/reaction in the qPCR plate.

2.4. Assessing gene expression variation using real-time PCR

To analyze the differential gene expression between the control and PFOA treated mice lungs we used quantitative real-time qPCR (StepOnePlus Real-Time PCR Systems; v 2.0 Applied Biosystems, Waltham, MA, USA). Primers for these genes were designed and ordered using PrimerQuest Tool (Integrative DNA Technologies, Inc., Commercial Park Coralville, IA, USA). Primer annealing temperatures were optimized using gradient PCR. Then, 3 μL of diluted cDNA and 13 μL of Powerup SYBR Green PCR master mix (primers added) (Thermo Fisher Scientific Inc., Waltham, MA, USA) were mixed in each well of the plate. Glyceraldehyde 3- phosphate dehydrogenase (*Gapdh*), a housekeeping gene was used as an exogenous reference to normalize transcription and the subsequent data were analyzed by the ΔΔCt method.

2.5. DNA bisulfite conversion and CpG pyrosequencing analysis

Genomic DNA from mouse lung samples were extracted per instruction of EpiTect® LyseAll Lysis Kit (QIAGEN, Germantown, MD, USA). Further, extracted DNA was bisulfite converted according to the protocol provided by EpiTect® LyseAll Lysis Kit (QIAGEN). Four sequences containing dense CpG islands within the promotor region of Tmprss2 were selected to investigate the DNA methylation status. Sequences on the antisense strand were examined and respectively provided below: 1. CGACCGTCTGACTGATTCGCCCGAGTGTACGT, 2. CGCGTGCGGTGACCCGAGGCGACGGTGCGGGCCCG, 3. CGGCGCTGCTGGGCCCTGGGGACCTTGGCAAGACGGGAATTGTCCGT, 4. AGGTTTGTCCGCAGTTCTCTTTGTTTCGGGAAAATCTCGGCAGTCGGGC. Both tem plate amplification and sequencing primers were acquired from GeneGlobe (QIAGEN). Templates were amplified with bisulfite converted DNA and PyroMark PCR kit (QIAGEN) using hot-start polymerase chain reaction (hsPCR). Amplified templates were processed and isolated with PryoMark Q24 Vacuum Workstation (QIAGEN). CpG methylation assays were performed on amplified templates with PyroMark Q24 Advanced CpG kit per manufacturer's instruction and adapted methods according

to [47,48] PyroMark Q24 Advanced (QIAGEN) pyrosequencing machine. All samples were run in duplicates to ensure consistency.

2.6. Quantification of PFOA accumulated in the lungs

PFOA extraction from the lung tissue (n = 3 mice per group) was performed following the method of Mamsen et al., 2017, along with modifications of this protocol [25,49]. In short, weighted lung tissues were homogenized in a ratio of 10 parts 70 % HPLC grade acetonitrile in DI water to one-part lungs tissue using Omni THQ—Digital Tissue Homogenizer for 2 min. Samples were vortexed for 30 min at room temperature, then centrifuged at maximum speed and the supernatant collected. Supernatant was placed in 2 mL autosampler tubes and injected into Ultra Performance Liquid Chromatography–Mass Spectrometry (UPLC–MS) using SYNAPT G2-Si system (Waters Corp., Milford, MA, USA). The mobile phase contained solvent A: 5 mM Ammonium acetate (NH4OAc·H2O) and solvent B: Acetonitrile. Calibration curve was developed using known concentrations of PFOA in 70 % acetonitrile. Electrospray ionization (ESI) in the mass spectrometry was run in negative ion mode for PFOA detection and the target *m/z* obtained was 412.96. Using the standard calibration curve and respective detection peak area for each sample, the amount of PFOA was calculated in µg/g of lung tissues.

2.7. Data processing and statistical analysis

Data analysis and figures were drawn in the OriginPro, Version 2021b. OriginLab Corporation, Northampton, MA, USA. Normally distributed data was analyzed using the analysis of variance (One-way ANOVA), whereas the not normally distributed data was analyzed through a non-parametric test, Mann-Whitney. Statistical values were considered significant at p-value ≤ 0.05 (denoted by "*") and less significant at p-value > 0.05.

3. Results

3.1. Accumulation of PFOA in lungs tissue

PFOA has high bioaccumulation characteristics in the body organs due to its slow elimination rate and metabolically inert characteristics. The PFOA level was measured in the lung tissue of the CD1 mice dosed with 5 and 20 mg/kg/day for a total of 10 days. The amount of accumulated PFOA in these groups along with vehicle control is shown in Fig. 1. The mean concentration of PFOA detected was 14.14 ± 2.95 and 36.41 ± 15.09 µg/g of lungs tissue in mice dosed at 5 and 20 mg/kg respectively. PFOA accumulated proportionally with an increase in dosing concentration. High accumulation in lungs suggest high affinity of PFOA for lung tissues and the associated adverse effects.

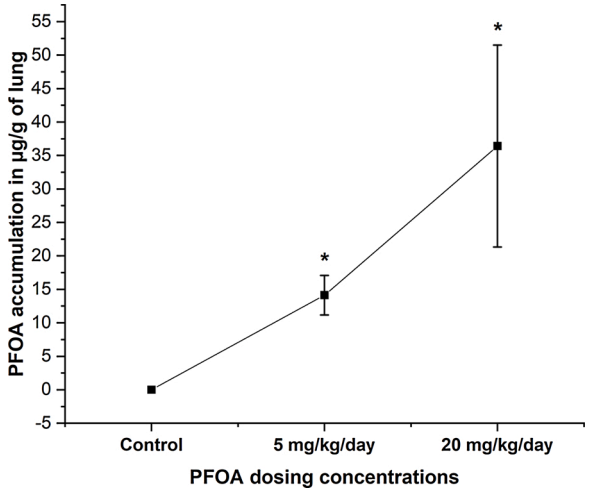

Fig. 1. Accumulation of PFOA in the lungs tissue of CD1 mice treated with vehicle control (0.5 % Tween 20 in water) or 5 and 20 mg/kg/day PFOA for 10 days. Accumulated PFOA is presented in µg/g ± standard deviation. Statistical values are considered significant at p-value ≤ 0.05 (denoted by "*").

3.2. Epigenetic changes in lungs

The DNA methylation is one of the key epigenetic mechanisms which regulates gene expression without changing the sequence. DNMT3a and DNMT3b members of methylation enzymes establishes methylation at new CpG sites where as DNMT1 retains the preexisted/inherited methylation [27]. In the current work we examined these 3 functional isoforms of DNMTs upon PFOA exposure.

In mice lungs exposed to PFOA, an overall decrease in expression patterns of *Dnmts* was noted. *Dnmt1* and *Dnmt3a* had lower gene expression at high PFOA concentration (20 mg/kg/day) while the expression of *Dnmt3b* decreased significantly at 5 mg/kg/day, as shown in Fig. 2A. Overall, a decrease in the expression of these regulatory genes suggests a possible role in decreased methylation in cells from lungs.

Next, we examined the expression of *Tets*, a class of demethylating enzymes which has a demethylating function contrary to the *Dnmts*. At the gene expression level there is an overall decrease in mRNA transcription of these demethylation genes. A significant decrease in *Tet1* and *Tet2* at 5 mg/kg PFOA and at 20 mg/kg in *Tet3* gene was noted (Fig. 2B).

3.3. Ace2 and Tmprss2 genes mRNA level increased with PFOA

ACE2 is the key membrane protein which has been implicated in different physiological and pathophysiological conditions of the human and other mammals. TMPRSS2, a member of serine protease family which fuse with the v-ets erythroblastosis virus E26 oncogene homolog (ERG) is reported to be dominant in the pathophysiology of prostate cancer [50]. In this study we analyzed the differential expression of these genes in PFOA treated and untreated mice. There is a dose dependent increase in the gene expression level of *Ace2* and *Tmprss2* genes in the lungs of these mice exposed to PFOA. *Ace2* mRNA level significantly increased at 20 mg/kg/day whereas *Tmprss2* expression was significantly enhanced at both the lower and higher PFOA dosing levels (Fig. 3).

3.4. Alteration in the Tmprss2 gene promotor methylation level by pyrosequencing

Since *Tmprss2* gene expression increased significantly in both low and high PFOA dosing, we tested the CpG methylation alterations in the promotor region of this gene and targeted 20 different CpG sites which clustered in 4 sequences. In 20 mg/kg/day PFOA treatment group, 6 out of 20 different CpG sites were significantly hypomethylated whereas 2 sites were hypermethylated (Fig. 4). In the 5 mg/kg/day treatment group, 3 sites were noted to have significant hypomethylation pattern and 1 site exhibited increased methylation as shown in Fig. 4. Overall significant hypomethylation pattern of target CpG sites in promotor region support the upregulation of *Tmprss2* in qPCR analysis.

4. Discussion

PFOA, the ubiquitous synthetic chemical contaminant in the environment enters the drinking water and food chain, to which human and other organisms are exposed frequently. Although PFOA is banned in US now, due to its abundant use in the past, high bioaccumulation, high stability, and long half-life the toxicant is bound to exist in the environment and water sources for a long period [1–3]. Few studies have reported on the toxic effects of PFOA in the lungs and no report exists on

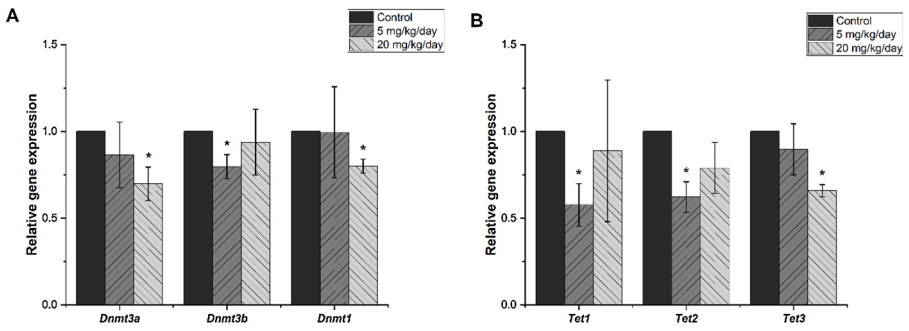

Fig. 2. Gene expression level changes in the DNA methylation regulators genes, *Dnmts* and *Tets* of the mice treated with 5 mg/kg and 20 mg/kg PFOA along with control. **(A)**. *Dnmt3A*, *Dnmt3B*, and *Dnmt*1 and **(B)**. *Tet1*, *Tet2*, and *Tet3* mRNA levels were down regulated. Gene expression is shown as fold change ± standard error mean (SEM) relative to control group. Statistical values are considered significant at p-value ≤ 0.05 (denoted by "*").

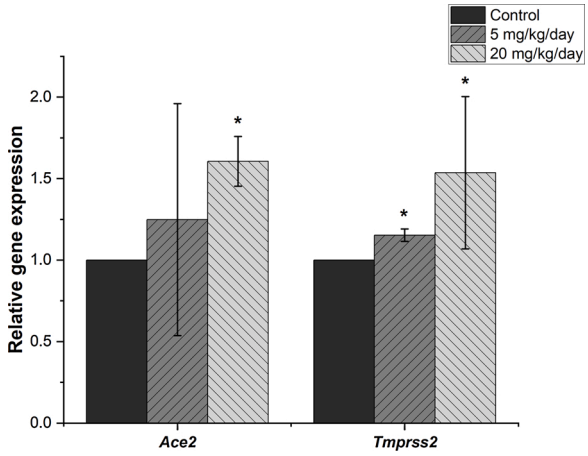

Fig. 3. Expression levels of the genes for key epithelial membrane proteins *Ace2* and *Tmprss2* in 5 mg/kg and 20 mg/kg PFOA treated groups *vs* control. Gene expression is shown as fold change ± SEM relative to control group. Statistical values are considered significant at p-value ≤ 0.05 (denoted by "*").

the alteration of epigenetic modulators and key epithelial cell receptors in lungs upon PFOA exposure. In the current work we evaluated accumulation of PFOA in lung and explored its effects on key epigenetic regulators and key membrane SARS-CoV-2 receptors.

4.1. PFOA exposure by oral route accumulate in lungs

Lung is one of the primary organs exposed to environmental contaminants directly through inhalation and indirectly through oral ingestion [51]. PFOA in drinking water and food is absorbed by the body from gut and distributed to different body organs. In our experiment, the orally administered PFOA accumulates in proportion with dosing concentration in lungs. Variation observed in PFOA accumulated in 20 mg/kg group is possibly due to the individual animal physiology, metabolism, and elimination capability. Prior studies have shown such a large variation in the accumulation of these PFAS compounds in different tissues, specifically in high treatment groups [6,19]. An animal model study showed that lung has the 3rd highest PFOA accumulation after liver and kidney in rats exposed to PFOA [19]. Autopsies of organs from humans showed that PFAS accumulation in the lungs is the highest, amounting to 29.2 ng/g of tissue weight [52]. Our current accumulation data support the previous reported level of PFOA in lungs. The high concentration of these contaminants can lead to pulmonary toxicity leading to other complications of the immune functions requiring further inquiry on the accumulation and role of PFOA specifically in lungs.

4.2. PFOA alters DNA methylation regulating enzymes in lungs

Epigenetic mechanisms regulate the development and differentiation of organs and their proper function by controlling gene expression without mutating the original DNA sequence [53]. To explore epigenetic mechanisms that result in gene expression alterations in lung tissues exposed to PFOA, we evaluated gene expression alterations in DNA methylation regulators. Epigenetic alterations regulate development of lungs and physiological functioning, leading to abnormal conditions that could potentially lead to disease [54]. Epigenetic toxicity due to PFOA is a less explored area specifically in tissues of the lungs where PFOA has the highest accumulation. Our recent studies have already reported on the alteration of epigenetic regulators such as DNMTs, TETs and Histone deacetylase enzymes in different mice organs and human cell lines [23–26]. Our current findings show significant alteration in the DNMTs enzymes expressing genes. A significant decrease in trend was observed in the genes responsible for these DNA methylating and demethylating enzymes in both low and high PFOA dosing circumstances. Pro-metastatic oncogene synuclein-γ was abnormally upregulated due to the downregulated *DNMT3B* in cigarette smoke exposed lung cancer cells based on *in vitro* studies [55]. DNMTs alteration has been reported in several tumorigenesis conditions delineating their role in the initiation and inhibition of cancer [56].

SARS-CoV-2 infection downregulated the expression levels of *DNMT1*, *DNMT3A*, and *DNMT3B* in lung epithelial cells [57]. We further noticed a significant decrease in the mRNA level of Tets expressing genes in the PFOA dosed mice groups. TETs enzymes are reported in several carcinomas and their lower expression is considered a hallmark of gastric, lung, prostrate, liver and breast tumors [58]. TET1 enzyme downregulation has been recently reported in the promotion and occurrence of hepatocellular and bladder carcinoma [59,60]. There is a similar decrease in the expression levels of these epigenetic genes based on our previous PFOA treatment studies which suggests possible downstream effects in pathways and mechanisms of these altered epigenetic modulators [25,26].

We observed that PFOA has downregulated expression of both *Dnmts* and *Tets* genes. This collaborative up or down regulation is possible and has already been observed in some diseased conditions and developmental process. For example, in hepatoblastoma patients *DNMTs* and *TETs* genes are upregulated compared to normal liver but downstream effects are only observed for high level of TETs enzymes [61]. Another recent mouse model study focused on the hypothalamus development which showed highest expression of all *Tets* and *Dnmts* genes irrespective of their methylation and demethylation activities [62].

4.3. PFOA upregulates expression levels of Ace2 and Tmprss2

ACE2 and TMPRSS2 are the key epithelial membrane proteins which are more frequently reported in different diseased conditions instead of

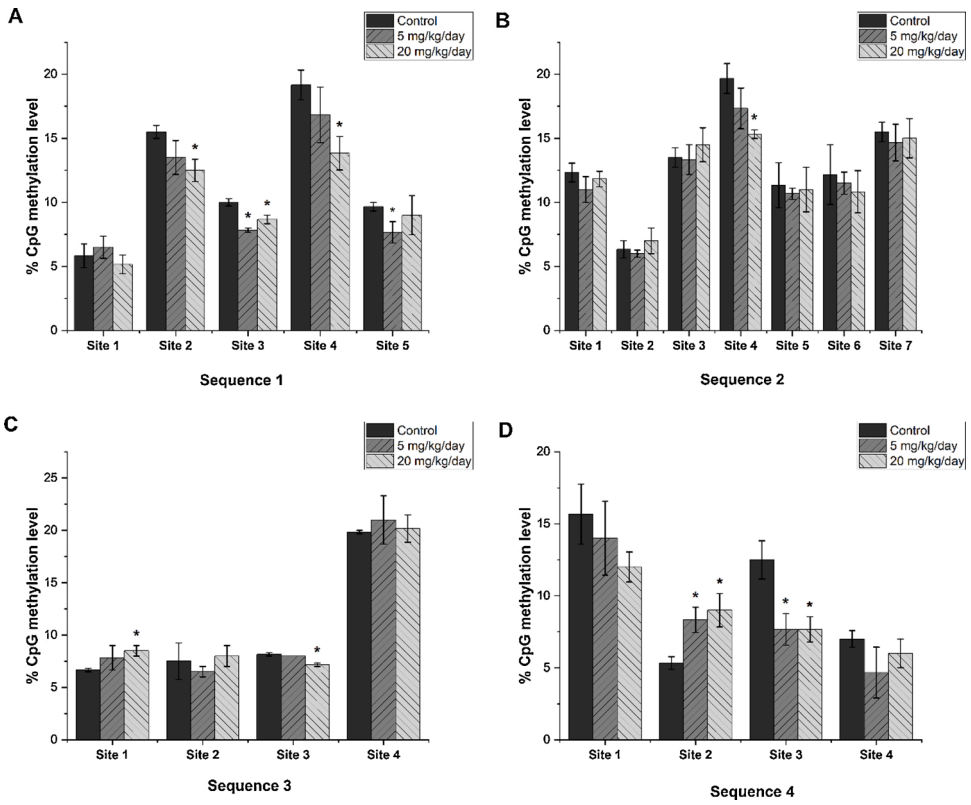

Fig. 4. Differential methylation pattern in the promotor region of *Tmprss2* gene in 5 mg/kg, 20 mg/kg PFOA treated and control mice. Target sequences (Sequence 1 – 4) have dense CpG sites in the *Tmprss2* promotor region. CpG methylation levels are shown in % ± SEM. Statistical values are considered significant at p-value ≤ 0.05 (denoted by "*").

their normal physiological functions. These genes are coregulated [63] and are highly expressed in testis, prostrate, aerodigestive tract and cardiovascular epithelial cells [64]. Our evaluation of *Ace2* and *Tmprss2* gene expression in mice lungs tissue showed significant change in expression among PFOA treated groups. *Tmprss2* expression level increased proportionally upon lower and higher exposure of PFOA. Recent reports showed upregulation of *ACE2* and *TMPRSS2* genes in smokers in comparison to non-smokers in both human and rodent models [35,65,66]. These genes were also upregulated in murine lungs with environmental pollutant: particulate matter which is a mixture of different ions, elemental and organic carbon, metals, and polycyclic aromatic hydrocarbons [67]. Our findings are in line with the previous reports and suggests a possible effect of the external environment on these genes.

These coregulated receptors in epithelial membrane paves the way for different viral infections, specifically the SARS-CoV-2. Spike protein of SARS-CoV-2 is recognized by the host and the virus binds to ACE2 receptors in human. Then TMPRSS2 protein cleaves the spike protein and the viral envelop fuses with host membrane and invades the cell [35]. Individuals with preexisting debilitating health conditions for example obesity, diabetes, cardiovascular implications, hypertension, chronic lung, liver, and kidney diseases are more prone to SARS-CoV-2 infection and comorbidities due to their higher *ACE2* expression [68]. In contrast children and infants with very low pulmonary *ACE2* expression are almost not affected by this viral infection [69,70]. This indicates that *ACE2* expression is directly proportional to the SARS-CoV-2 infection and fatalities. Overexpression of these genes with PFOA accumulation in the lungs increases their susceptibility to SARS-CoV-2 and potentially other viral infections from the SARS family.

Further evaluation of the promotor region of *Tmprss2* gene showed differential CpG methylation pattern at almost 33 % of our target sites in PFOA treated *versus* control group. Higher level of DNMT1 is linked to hyper-methylation and downregulation of *TMPRSS2* gene in androgen receptor negative prostate cancer cells [71]. *Dnmt1* is downregulated in our case which supports the link between this epigenetic regulator and *Tmprss2* methylation pattern and expression. 30 % of our target CpG sites in the promotor region of *Tmprss2* had significantly reduced methylation after the highest PFOA dosing. *Ace2* and *Tmprss2* genes have been reported to be epigenetically modulated by DNA methylation patterns in the promotor regions [42,43]. In our current work although the downregulation of *Dnmts* support the upregulation data for the *Ace2* and *Tmprss2* genes there could be several other responsible factors involved and the need for further studies to explore the exact mechanism is imperative.

In conclusion, PFOA has significant effect on DNA methylation regulators and key membrane proteins Ace2 and Tmprss2. Alteration in the expression profiles of the target genes is potentially due to the higher amount of PFOA accumulation in these lung tissues. There is a need for future research to categorically assess these initial findings at the protein level by knockout models. Current work provides a baseline to evaluate other PFAS compounds and environmental toxicants and their effects on our health and our vulnerabilities to viral infections, specifically SARS-CoV-2 amid exposure to these contaminants. Future large scale epidemiological studies should be designed to further evaluate the possible association between PFAS accumulation in vulnerable populations and SARS-CoV-2 infectiousness and fatalities.

Data availability

Not applicable.

Availability of data and materials

Not Applicable.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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