**The impact of vaping on adolescent lung function and nasal epithelium gene expression.**

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**Abstract**

**Background:** Electronic cigarettes (e-cigs) are touted as safer alternatives to traditional tobacco products; however, there are no substantive data to corroborate this claim. Vaping among youth is a pressing public health problem and is a risk factor for negative health effects. We aimed to investigate the impact of vape exposure on lung function, nasal epithelial gene expression in adolescents.

1. **Research Question: Does** vape use result in decreased lung function and modified nasal epithelial gene expression?

**Study Design and Methods:** We performed focus groups and interviews of 50 middle and high school students at high school and community-based settings in Colorado to explore youth perceptions on vaping. Participants completed focus groups and confidential surveys on their vaping use. Airflow obstruction was investigated using impulse oscillometry (IOS) and spirometry. Nasal epithelial brushing was collected for gene expression analyses. RNA-sequencing was performed using the Illumina HiSeq platform. We compared IOS measures between vaping and non-vaping groups using Mann-Whitney U tests. Differential expression testing was performed in DESeq2 and pathway enrichment was performed using Gene Set Enrichment Analysis (GSEA). Written informed consent was obtained from participants and Institutional Review Board approval was obtained.

**Results:** Fifty participants were recruited. Vaping subjects were defined as those who reported vaping within the past 6 month while non-vaping (hereafter control) subjects did not report any vape exposure in the past 6 months. The mean (standard deviation) age was 15.1 (1.5) years for vaping subjects and 14.5 (1.4) years for control subjects. We found significant differences in airway resistance by vaping status. Mean airway resistance identified by the IOS R5 measure revealed that vapers (n=13) had higher values than control subjects (n=37) [p=0.016, Figure 1], an early indication of increased airflow obstruction. Of 16860 nasal epithelial genes tested, 7136 were significantly differentially expressed between vaping and control subjects (false discovery rate adjusted p-value FDR < 0.05), after adjusting for covariates (age, sex, recruitment center, and two RUVSeq components). Pathway enrichment analyses identified overexpression of inflammatory response genes and underexpression of genes associated with ciliogenesis in the vaping group compared to controls.

**Interpretation:** Our results suggest that vaping results in increased airway resistance and dysresgulation of nasal epithelium genes, including genes involved in airway inflammation and ciliary function. Furthermore, dysregulation of these gene expression pathways may be a biological mechanism for the development of increased airway resistance due to vaping even during adolescence.

**Clinical Trial Registration:** N/A

1. **Background**

The increasing use of Electronic Nicotine Delivery Systems (ENDS), such as electronic cigarettes (e-cigs), is a significant and emerging public health problem [1]. ENDS represents a diverse class of products such as electronic cigarettes, vapes, vaporizers, vape pens, hookah pens, and pods [2], and exposure to ENDS aerosols depends on the user and device characteristics [2]. They may contain nicotine and can be classified as tobacco products [3]. Herein, we focus on one type of ENDS, namely e-cigs, the use of which we term vaping. E-cigs are touted as safe alternatives to traditional tobacco products, however, there is no substantive data to corroborate this assertion [4]. The solutions in e-cigarettes, and their resulting aerosols, contain nicotine, carcinogens, and metal particles, to which users and nonusers in close proximity can be exposed [3]. The youth and young adults are the predominant population using e-cigarettes and are at the greatest risk to the negative effects of nicotine exposure [5]. Given the attractive flavors, marketing and design, and its appeal to youth, e-cigarettes has the potential to reverse decades of progress achieved in nicotine and tobacco product use [6].

While traditional cigarette smoking has declined, e-cig usage has increased, attracting both former, current and never tobacco smokers [1]. Vaping among youth is a pressing public health issue, with prevalence of use surpassing that of tobacco cigarettes [7]. There is reported increase in past-30-day e-cigarette use among high school students. Particularly, the National Youth Tobacco Survey reported increased from 11.3% in 2017 to 20.8% in 2018 [8]. Monitoring the Future Study also reported increased use from 11.0% in 2017 to 20.9% in 2018 [9]. A recent study on failed nicotine quit attempts, among US adolescent e-cig and traditional cig users, shows that levels are back to where it was 13 years ago [10]. Certainly, the contribution of e-cigs to unsuccessful nicotine quit attempts among adolescents is substantial and warrants urgent attention.

Furthermore, increased use of vaping has been identified specifically among minority youth at alarming rates much higher than non-minorities. Hispanic/Latino youth are reported to have increased rates of vaping compared to non-Hispanic/Latino youth [7]. Colorado is a leader in electronic smoking device use among teenagers [11]. In Colorado 48.3% of Latino high school youth reported ever having vaped and recent national reports showing disproportionately higher e-cigarette use among Latino youth [12]. For instance, among minority race-ethnic groups, 25.4% Hispanics were reported to regularly use e-cigarettes compared to 16.9% and 17.2% Blacks and Asians respectively [11]. Indeed, there is urgent need for sound science and policies that will promote a tobacco-free lifestyle for current and future generations.

Incidentally, vaping is not harmless. There are pathophysiologic impacts that have been reported on the human pulmonary system.Tobacco smoke is known to be highly proinflammatory and has been shown to trigger the release of inflammatory cytokines and other biological changes, including goblet cell metaplasia and neutrophil influx [13], however the impact of e-cig aerosols in the long term is not clear. The solutions found in e-cigarettes present a novel mixture of chemicals, including flavors and sweeteners designed to mask nicotine's bitter taste [1]. Thus, while nicotine is known to adversely alter airway physiology, the effects of these novel chemical mixtures, either by itself, and/or together with nicotine have not been studied. Short-term exposure to e-cigarettes in healthy adults increases airway resistance, with some evidence demonstrating decreased lung function after exposure [14]. However, what happens in the long term during a critical development window such as adolescence, is unknown.

Given the paucity of information on the effects of vaping on the lungs in the long-term, the high-risk youth population with access to these devices, and the current epidemic, we pilot tested this study to help address these knowledge gaps. By presenting evidence on biologic determinants of reduced lung function we will fill in a critical knowledge gap on the health effects of vaping in the human population. Thus, we aimed to investigate the impact of vape exposure on measures of lung function in adolescents and to compare nasal epithelial gene expression and DNA methylation in those who vape to non-vaping controls to determine the biological impact of vape exposure. The motivating hypothesis for this work is that vape exposure is associated with abnormal lung function measures and that nasal epithelial gene expression would be modified by vape exposure. To address this hypothesis, we sought to answer the following questions: Q1) what is the impact of vape exposure on measures of lung function and Q2) Are nasal epithelial genes differentially expressed depending on vaping status?

**Methods**

**Study Participants**

Study participants were enrolled in a pilot study aimed to examine vaping initiation and its respiratory effects among the youth in Colorado. Adolescent participants completed focus groups on reasons for vaping initiation and confidential surveys on their vaping use. Adolescents from high schools in Pueblo, Aurora and Denver were enrolled in this pilot study. Specifically, youth aged 12 to 17 who attend a public school in Pueblo County were eligible as participants while those who did not given consent or who had underlying nasal mucosal abnormalities were excluded. Written informed consent was obtained from participants. We used Research Electronic Data Capture (REDCap) to securely enter and store data. The Institutional Review Board at the University of Colorado Anschutz Medical Campus approved the current study.

**Vape use (or vaping) variable definition**

To assess vape use in this study, we used self-reported vape status derived from answers provided by participants on the following three survey questions:

1. “Have you ever vaped?”
2. “How many times have you vaped in the last 30 days?”
3. “When was the last time you used a vape device?”

These three questions were used to construct a dichotomous variable which defines subjects who have vaped in the last 6 months. This variable will be referred to as Vape Status. One participant reported that they had used a vaping device 5 out of the last 30 days but did not respond to the question on the last time the participant vaped. This participant is recorded as “vaped” in this analysis.

**Outcome variable 1: Lung function measurement**

Impulse oscillometry (IOS) and spirometry measurements were obtained from each participant. Since IOS permits passive measurement of lung mechanics, it can identify small airway obstruction and is more sensitive than spirometry for peripheral airway disease [15, 16]. Using tremoflo, a portable IOS analyzer (Thorasys INC, Montreal, Canada), forced oscillation measurements (FOT) measurements were applied in 60 second measurements in triplicate, capturing reactance (XR), resistance (R), resonance frequency (XA). Data were captured within 5–37 Hz [17]. Additionally, we performed spirometry at each visit following the American Thoracic Society/European Respiratory Society guidelines [18]. Calibration and quality control are followed according to the manufacturer’s recommendations following European Respiratory Society guidelines IOS [19].

**Outcome variable 2: Nasal epithelium gene expression**

**Nasal epithelium sample collection**

Nasal epithelial brush specimens from study participants were obtained from the anterior nasopharynx using a sterile cytology brush under the inferior turbinate and under direct visualization using a sterile nasal speculum (Welch Allyn, Skaneateles Falls, NY). Due to the COVID-19 pandemic, samples were placed in DNA/RNA Shield (Zymo Research, Irvine, CA) to ensure appropriate viral inactivation. The cytology brush was placed into the nasal epithelial cell collection tube with RNALater (ThermoFisher Scientific, Waltham, MA). The brush was then cut off its handle and stored at -80°C.

**Nasal epithelium gene expression analysis**

RNA sequencing (RNA-seq) was conducted using the Illumina HiSeq platform at the Genomics Core at the University of Colorado. RNA was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA). Samples were assessed for yield and quality using the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and Qubit fluorometry (Thermo Fisher Scientific, Grand Island, NY).

To minimize potential batch effects, all samples were submitted together for RNA-seq. Sequencing libraries will be prepared using the standard TruSeq RNA Sample Prep Kit v2 Protocol (Illumina, San Diego, CA). mRNA libraries will be sequenced on the Illumina NovaSeq 6000 platform with a per-sample target of 50 million 100 bp paired-end reads (Illumina, San Diego, CA). We examined the quality of the sequencing reads (FastQC[20]) and removed adaptors and low quality base calls (Cutadapt [21]). Sequences were aligned to the human genome (GENCODE GRCh38) [22] and converted to the count of reads overlapping with each gene using STAR [23]. We performed quality control at the sample and gene-level, retaining genes detected with average counts per million > 0.5 for further analyses. We examined individual genes for quality, retaining genes detected with >10 counts in at least 80% of all samples for further analyses. Finally, we applied the voom [24] transformation to the counts, yielding an approximately normally distributed measure of expression for each quality-checked gene.

To ensure accuracy of our data, our top 10 genes that were differentially expressed by vape exposure (inflammatory genes n=5, ciliary dysfunction genes n=5), were validated in nasal epithelium using QuantStudio TaqMan OpenArray platform (Life Technologies, Carlsbad, CA), using RNA-specific primers for reverse transcription of the RNAs (MegaPlex RT Primers) followed by qPCR using primers and TaqMan MGB probes. The resulting qPCR measurements were expressed as ΔCT values [25], representative of the expression of the target gene normalized to endogenous housekeeping genes. We also run 10% technical replicates to ensure reproducibility of our findings.

**Covariates**

### For adjusted analyses, we include the following the covariates into the models, as these have been identified as potential confounders in the literature: 1) Geographic Location – *subjects’ geographic location,*city*, was grouped into the new broader variable termed* recruiting centerwhich encompasses the broader geographic region where they live 2) Sex 3) Age 4) Latinx ancestry.

**Statistical Analysis**

We examined differences in demographic measures among vaping and non-vaping study participants using *χ*2tests for categorical variables and two-sample t-tests for continuous variables. Next, we conducted a series of bioformatic analysis to evaluate associations between vape status and lung function and gene expression among study participants.

For the lung function data, spirometry and IOS measures were visually inspected for normality using histograms before conducting association studies with vaping status.

Gene expression data

Normalization  
To normalize our data, we used the *RUVr* from the R package RUVSeq. *RUVr* uses the deviance residuals from a first pass negative binomial GLM to perform a factor analysis which corrects for unwanted technical effects. The first-pass model formula is presented below:

raw read count∼β0+β1∗vape status +β2∗male +β3∗ageraw read count∼β0+β1∗vape status +β2∗male +β3∗age

*RUVr* was performed k = 2 factors. The best k for factor analysis was determined visually using an elbow plot, RLE plots, and dendrograms. This analysis used *edgeR* to fit the first pass GLM due to its reference in the literature for the *RUVr* procedure mentioned above (CITE).

Gene counts were fit using negative binomial models from the R package *DESeq2*. To account for multiple testing the False Discovery Rate (FDR) was calculated, and significance set at FDR < 0.05.

To parse out the effects that vape status and recruitment center have on gene expression, the following models were fit and compared using Likelihood Ratio Tests (LRTs) in DESeq2:

Full Model:

raw read count∼vape status+recruitment center+sex+age+ruv1+ruv2raw read count∼vape status+recruitment center+sex+age+ruv1+ruv2

Reduced Model (testing vape status and recruitment center): raw read count∼ sex+age+ruv1+ruv2raw read count∼ sex+age+ruv1+ruv2

Reduced Model (testing vape status):

raw read count∼recruitment center+sex+age+ruv1+ruv2raw read count∼recruitment center+sex+age+ruv1+ruv2

Reduced Model (testing recruitment center):

raw read count∼vape status+sex+age+ruv1+ruv2raw read count∼vape status+sex+age+ruv1+ruv2

Likelihood Ratio Tests (LRTs) were used to compare full and reduced models. All LRTs used the same full model, which included vape status (binary Y/N) and recruitment center (3-level categorical), sex (binary M/F), age (continuous), and two normalization factors from the RUVr Analysis. In each scenario above, the LRT examines if the additional covariates included in the full model and not the reduced model significantly increase model fit. The reduced model for vape status and recruitment center tests if vape status and recruitment center (together) significantly explain gene expression after adjusting for sex, age, and the two RUV factors. The other models test similarly, but only for one covariate at a time. The model we are most interested in is the reduced model testing only vape status. The others were included to investigate the presence of recruitment center bias in the data.

**Sensitivity analysis**

Finally, to check the robustness of our results, we conducted a sensitivity analysis by limiting the recruitment center to Pueblo (where 12/13 vapers were recruited). We report result in terms of p-values, effect sizes and 95% confidence intervals. All statistical tests were two-tailed, and p ≤ 0.05 and FDR < 0.05 were considered statistically significant for lung function and gene expression analyses. respectively.

To assess the difference in model outputs, the following model was fit in DESeq2 with all the recruited subjects and then with the subjects from the Pueblo recruitment center.

Full Modelraw read count∼vape status+recruitment center+sex+age+ruv1+ruv2

Reduced Model (Vape Status) raw read count∼recruitment center+sex+age+ruv1+ruv2

Likelihood Ratio Tests (LRTs) were implemented to test for gene significance and corrected with FDR ≤ 0.05. When fitting the model for only the subjects recruited in Pueblo, the *recruitment center* covariate is held constant, and therefore, not included in the model. Each of these models will test if *vape status* contributes significantly to gene expression.

To assess the difference in the models, the results will focus on genes with relatively low variability. To achieve a stable subset, a cutoff value of |Log2(Fold-Change)| > 2 was be used. All analyses performed using R version 4.2.1

**Pathway enrichment analysis**

To identify differentially enriched biological pathways, Gene Set Enrichment Analysis (GSEA) was conducted after obtaining differential gene expression results.

GSEA requires the input of both a ranked list of genes and a list of biological pathways with their associated genes. For ease of comparison with various pathways, genes were mapped from ENSEMBL IDs to ENTREZ (NCBI) IDs. GSEA analysis was conducted using the R package *fGSEA* ver. 1.23.0.

**Ranks**  
A filter for |log2(FC)|>2 was applied as in previous sensitivity analyses. Ranks were calculated as:

Rank=sign(log2(FC))∗−(log10(p-value))

using the fold-change estimate and associated p-value from differential expression analysis[1](file:///C:\Users\scommod\Desktop\AGOLD\Vaping%20manuscript\November%202022%20Vaping%20Manuscript\Sharma_Vape_Results_2022_10_26\Sharma_Vape_Results_2022_10_26\GSEA_Report_2022_10_26.html#ref-xiao2012).

**Pathways**  
We completed GSEA for the following pathway collections:

1. Reactome
2. Gene Ontology (GO)
3. Koyoto Encyclopedia of Genes and Genomes (KEGG)

Only pathways with 2 or more ENTREZ genes were considered for GSEA. After the initial analysis, we determined redundant pathways using the function *collapsePathways* from fGSEA[3](file:///C:\Users\scommod\Desktop\AGOLD\Vaping%20manuscript\November%202022%20Vaping%20Manuscript\Sharma_Vape_Results_2022_10_26\Sharma_Vape_Results_2022_10_26\GSEA_Report_2022_10_26.html#ref-korotkevich2016)(CITE). This report refers to non-redundant pathways as ‘independent’ pathways throughout the report. It should be noted that this terminology refers only to the idea that some gene-sets are represented in multiple pathways. In this case, independence does **not** refer to biological relationships between pathways, but a statistical determination. All pathways were retrieved from Ensembl (ver. 106) using biomaRt ver. 2.53.2[2](file:///C:\Users\scommod\Desktop\AGOLD\Vaping%20manuscript\November%202022%20Vaping%20Manuscript\Sharma_Vape_Results_2022_10_26\Sharma_Vape_Results_2022_10_26\GSEA_Report_2022_10_26.html#ref-cunningham2021)(CITE).

* + - 1. **Results**
      2. *Descriptive statistics*
      3. A total of 50 subjects participated in the study.  Vaping subjects were characterized as adolescents who reported vaping within the past 6 months (n=13) while controls subjects were adolescents who did not have any vape exposure in the past 6 months (n=37). The mean (standard deviation) age was 14.8 (1.4) years for vaping subjects and 14.6 (1.4) years for control subjects. We observed some demographic differences by vaping status. Most vaping subjects were recruited in Pueblo (91%) and identified as LatinX (85%). 53% of subjects were female. Spirometry measurements were missing for all but one of the vaping subjects and IOS data was available for most subjects (Table 1).
  1. **Table 1. Demographic characteristics and lung function testing results of study participants**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Did Not Vape in Last 6 Months (N=37)** | **Vaped in Last 6 Months (N=13)** | **Total (N=50)** |
| **Sex** |  |  |  |
| Female | 17 (45.9%) | 8 (61.5%) | 25 (50.0%) |
| Male | 20 (54.1%) | 5 (38.5%) | 25 (50.0%) |
| **Age (yrs)** |  |  |  |
| Mean (SD) | 14.6 (1.4) | 14.8 (1.4) | 14.6 (1.4) |
| Range | 12.0 - 17.0 | 13.0 - 17.0 | 12.0 - 17.0 |
| **Recruitment Center** |  |  |  |
| Aurora | 15 (40.5%) | 0 (0.0%) | 15 (30.0%) |
| CommCity/Denver | 13 (35.1%) | 1 (7.7%) | 14 (28.0%) |
| Pueblo | 9 (24.3%) | 12 (92.3%) | 21 (42.0%) |
| **Ethnicity** |  |  |  |
| LatinX | 23 (62.2%) | 11 (84.6%) | 34 (68.0%) |
| Non-LatinX | 14 (37.8%) | 2 (15.4%) | 16 (32.0%) |
| **FEV1** |  |  |  |
| N-Miss | 10 | 12 | 22 |
| Mean (SD) | 2.6 (0.7) | 3.9 (NA) | 2.6 (0.7) |
| Range | 1.2 - 3.9 | 3.9 - 3.9 | 1.2 - 3.9 |
| **FEV1/FVC (%)** |  |  |  |
| N-Miss | 10 | 12 | 22 |
| Mean (SD) | 0.8 (0.1) | 0.7 (NA) | 0.8 (0.1) |
| Range | 0.5 - 1.0 | 0.7 - 0.7 | 0.5 - 1.0 |
| **R5** |  |  |  |
| N-Miss | 1 | 0 | 1 |
| Mean (SD) | 4.0 (0.9) | 5.0 (1.3) | 4.3 (1.1) |
| Range | 2.0 - 6.1 | 3.7 - 7.6 | 2.0 - 7.6 |
| **X20** |  |  |  |
| N-Miss | 4 | 2 | 6 |
| Mean (SD) | 0.1 (0.6) | 0.7 (0.9) | 0.2 (0.7) |
| Range | -1.1 - 2.4 | -1.0 - 2.3 | -1.1 - 2.4 |

***Lung function measures***

To assess the impact of vape exposure on measures of lung function (outcome variable 1), *[Need TRENT to test association between vape status and lung function measures and provide some p value]* we tested the association of vape exposure with IOS measurements using linear models adjusted for age and sex. We observed significant differences in airway resistance by vaping status. Figure 1 depicts pulmonary function (FEV1/FVC) and IOS (R5 and X20) measures. FEV1/FVC was completed by only 22 individuals while R5 and X20 represent n = 49 and n = 44 participants, respectively.

Mean airway resistance (R) values calculated over a measurement period of 60 seconds at a frequency of 5 Hz (R5) revealed differences between vaping (n=13) and control (n=37) subjects. When vaping subjects are compared with controls, there was increased airway resistance (p=0.026) [Fig 1], an early indication of increased airflow obstruction. Additionally, our results showed higher X20 (reactance) values in vapers compared to non-vaping controls (p=0.016), which suggests adverse effects of vaping on lung parenchyma. Thus, these data are the first to demonstrate that vape exposure is associated with abnormal lung function even in early adolescence and shows the detrimental impact of vape exposure during a critical window of lung function development.

1. Chart, box and whisker chart

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2. **Fig 1. Differences in spirometry and impulse oscillometry (IOS) derived** **measures between vaping and control subjects.**

***Gene expression***

To assess our second outcome of interest, nasal epithelial gene expression was utilized to assess the biological impact of vape exposure on the airway epithelium of the study subjects.

When demographic and gene expression data were merged, there were a total of47 participants with at least demographic or genetic data. Three participants were missing genetic data and are excluded from subsequent analysis.

There were 60,651 genes before filtering out poor-quality genes. Of 16860 nasal epithelial genes tested, 7136 were significantly differentially expressed between vaping subjects and control subjects (false discovery rate [FDR] adjusted p-value<0.05), after adjusting for age (years), male sex, recruitment center, and two inferred covariates (RUVr factors) [26]. A total of 4193 genes were underexpressed (e.g., *WNT5B*, *WNT3A, ACE2, IL7*) while 2943 were overexpressed (e.g. *TNF*, *MUC5A, IL10, IL17C*). Using a fold change cut of ≥2, there were 135 genes that were upregulated and 370 genes that were downregulated when vapers are compared with control subjects (Supplementary Table 1). Based on our top twenty differentially expressed genes, vaping subjects tended to have dysregulated expression of pathways associated with ciliogenesis and inflammation compared to the control group (Table 2).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Table 2. The top twenty transcripts with the greatest fold-change (log2FC) when adolescents who vaped in the last 6 months are compared to adolescents who did not vape. The fold-change is the expression in the vaping group relative to the control group (i.e., FC > 1 represents increased expression).** | | | | | | |
|  | **ENSG** | **Symbol** | **Log2FoldChange** | **p-value** | **FDR** | **Associated function** |
| 1 | ENSG00000147647.13 | *DPYS* | -3.87427 | 5.84E-06 | 4.17E-05 | AGE-RAGE signaling pathway |
| 2 | ENSG00000198838.14 | *RYR3* | -3.49922 | 9.22E-04 | 0.003522 | 9+2 motile cilium |
| 3 | ENSG00000162782.16 | *TDRD5* | -3.44945 | 0.001112 | 0.004156 | 9+2 motile cilium |
| 4 | ENSG00000152779.14 | *SLC16A12* | -3.38035 | 8.71E-09 | 1.25E-07 | 9+0 motile cilium |
| 5 | ENSG00000039537.14 | *C6* | -3.36715 | 0.004554 | 0.01382 | ADORA2B mediated anti-inflammatory cytokines production |
| 6 | ENSG00000280780.2 | *JAKMIP2-AS1* | -3.31596 | 8.15E-12 | 2.57E-10 | Novel transcript |
| 7 | ENSG00000244067.3 | *GSTA2* | -3.26412 | 1.71E-13 | 8.32E-12 | 9+0 motile cilium |
| 8 | ENSG00000260951.2 | *AC005100.1* | -3.16087 | 1.46E-05 | 9.43E-05 | Novel transcript |
| 9 | ENSG00000277893.2 | *SRD5A2* | -3.08175 | 1.37E-08 | 1.86E-07 | 9+0 motile cilium |
| 10 | ENSG00000268566.6 | *AC100781.1* | -3.0775 | 6.48E-04 | 0.002595 | Novel transcript |
| 1 | ENSG00000177257.3 | *DEFB4B* | 7.238958 | 0.002137 | 0.007273 | ADORA2B mediated anti-inflammatory cytokines production |
| 2 | ENSG00000198692.10 | *EIF1AY* | 6.434305 | 0.004871 | 0.014636 | 9+0 motile cilium |
| 3 | ENSG00000129824.16 | *RPS4Y1* | 6.369483 | 0.004625 | 0.013983 | ADORA2B mediated anti-inflammatory cytokines production |
| 4 | ENSG00000012817.16 | *KDM5D* | 5.943467 | 0.002072 | 0.00708 | 9+2 motile cilium |
| 5 | ENSG00000067048.17 | *DDX3Y* | 5.728893 | 2.07E-04 | 9.64E-04 | 9+2 motile cilium |
| 6 | ENSG00000114374.13 | *USP9Y* | 4.83661 | 0.005822 | 0.016994 | 9+0 motile cilium |
| 7 | ENSG00000232177.1 | *MTND4P24* | 4.667673 | 4.18E-04 | 0.001772 | Novel transcript |
| 8 | ENSG00000225972.1 | *MTND1P23* | 4.19136 | 9.33E-04 | 0.003559 | Novel transcript |
| 9 | ENSG00000201321.1 | *RNA5S9* | 3.742448 | 0.0038 | 0.011881 | Novel transcript |
| 10 | ENSG00000183878.15 | *UTY* | 3.451779 | 1.46E-04 | 7.11E-04 | 9+0 motile cilium |

Chart

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**Figure 2** 7136 differentially expressed between vaping subjects and control subjects (false discovery rate [FDR] adjusted p-value<0.05), after adjusting for age (years), male sex, recruitment center, and two inferred covariates (RUVr factors)

Chart, box and whisker chart

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**Figure 3 Differential expression of select genes between vaping and control subjects. These genes were selected by filtering for only genes with |log2fc| > 2 then sorted by their p-values to define the ‘top’ up- and down-regulated genes**

***Sensitivity analysis***

With 92% (12/13) vapers and 42% 21/50 participants recruited at the Pueblo center, we sought to understand how this demographic imbalance could act as a potential confounder or source of multicollinearity. As such we restricted analysis to participants recruited from the Pueblo center and compared it to the overall model containing all study participants to assess differences in estimates and significant gene composition between the models. We refer to the model containing all subjects as *Model A* and the model with only participants from the Pueblo recruitment center as *Model P*.

The top 2000 significant genes in models A and P are identical both before and after the selected cutoff value (|Log2(Fold-Change)| > 2). Figure 4 shows the breakdown of the significant genes in Models A and P after applying the cutoff. From the figure, there is a small amount of variability present in the estimates of the top 2000 genes when comparing difference in Log2(Fold-Change) between Models A and P; however, most of the estimates are clustered around the line representing equal estimates (red dotted line).

Chart, scatter chart

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**Figure 4. Significant genes from the model containing all study participants (*Model A)* and the model with only participants from the Pueblo recruitment center (*Model P)*.**

**Table 3: Top 10 Significant Genes when comparing statistical models with all study participants (Model A) and study participants from Pueblo only (Model P).**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Ensemble ID** | **Gene Name** | **Gene Type** | **Log2(FC) (Mod A)** | **Log2(FC) (Mod P)** | **Log2(FC) Difference (P - A)** | **Log2(FC) Difference (%)** | **FDR (Mod A)** | **FDR (Mod P)** |
| ENSG00000188039 | *NWD1* | protein coding | -2.156 | -2.21 | -0.055 | 2.542 | < 0.001 | < 0.001 |
| ENSG00000118997 | *DNAH7* | protein coding | -2.071 | -2.307 | -0.236 | 11.391 | < 0.001 | < 0.001 |
| ENSG00000105519 | *CAPS* | protein coding | -2.011 | -2.196 | -0.185 | 9.18 | < 0.001 | < 0.001 |
| ENSG00000287189 | *AL121956.6* | lncRNA | -2.825 | -3.101 | -0.276 | 9.765 | < 0.001 | < 0.001 |
| ENSG00000174844 | *DNAH12* | protein coding | -2.232 | -2.625 | -0.394 | 17.637 | < 0.001 | < 0.001 |
| ENSG00000154479 | *CCDC173* | protein coding | -2.422 | -2.639 | -0.217 | 8.956 | < 0.001 | < 0.001 |
| ENSG00000163263 | *C1orf189* | protein coding | -2.144 | -2.425 | -0.281 | 13.094 | < 0.001 | < 0.001 |
| ENSG00000197653 | *DNAH10* | protein coding | -2.381 | -2.675 | -0.293 | 12.317 | < 0.001 | < 0.001 |
| ENSG00000186976 | *EFCAB6* | protein coding | -2.243 | -2.47 | -0.227 | 10.107 | < 0.001 | < 0.001 |
| ENSG00000172955 | *ADH6* | protein coding | -2.852 | -3.266 | -0.414 | 14.52 | < 0.001 | < 0.001 |

These results demonstrate that estimates systematically decrease when looking only at subjects from Pueblo. That effect does not make a large difference in the number of significant genes or the direction of their effect. Since excluding those samples does not change the composition or direction of Log2(Fold-Change) estimates, further analyses included n = 47 subjects.

***Pathway enrichment analysis***

We tested 109, 174 and 668 total pathways tested for KEGG, Reactome and GO pathways respectively. Total genes matched to **any** tested pathway were 76, 110 and 542 for the aforementioned pathways respectively. There were 12, 18 and 40 **statistically** independent pathways at the FDR < 0.05 level. In tables 4-6, we present the top 15 pathways that were enriched.

**Discussion**

Our results suggest that adolescent vape exposure is associated with increased airflow obstruction and increased expression of inflammatory genes in the nasal epithelium of subjects who vape. Our work provides critical evidence of the negative impact of vape exposure on respiratory outcomes in adolescents.

Our findings are consistent with existing literature in adults demonstrating that short-term exposure to e-cigs increases airway resistance and decreased measures of lung function after exposure [14]. Our lung function data revealed significant differences in IOS but not spirometric measurements. To date, there have been few studies in humans looking at e-cig use and lung function measures and none have looked at long term exposures and their effects on lung function. One study reported acute effects on airway physiology and respiratory symptoms in COPD smokers, asthmatic smokers, "healthy" smokers and healthy never smokers after e-cig use. Significant findings in their results was that there was increase in airways resistance (ΔRaw) was detected in asthmatic (p=0.034) and healthy smokers (p=0.004). Even more surprising was an increase in Raw in never smokers after using e-cigs with (p<0.005) or without (p<0.001) nicotine [27]. Another study on documented e-cig exposures revealed adverse changes to respiratory metrics and markers of inflammation after a 5 minute e-cig use session. The authors observed that R5, a marker of total airway resistance, had increased post e-cig use in both healthy controls and mild asthmatics. This increase was significantly higher among the mild asthmatics [28].

The impacts of e-cig use on airway physiology have also been documented during passive exposures. In a repeated measures controlled study with 15 subjects, there were no significant changes in FEV1/FVC during a brief session of active e-cigarette smoking (indicative: 3% reduction in FEV1/FVC) nor a one hour passive e-cigarette smoking (indicative: 2.3% reduction in FEV1/FVC) [29]. A cross-over study also looked at 40 healthy nonsmokers (18–35 years old) exposed to exposed to e-cig emissions produced at two resistance settings, 0.5 ohm and 1.5 ohm. At the 1.5 ohm session, R5 showed a post exposure decrease trend that was not significant (0.39 pre to 0.38 kPa/L/s post exposure) [30]. A 2022 systematic analysis of the effects of e-cigs on lung function when compared to traditional cigarettes found that there were statistically significant increases in flow resistance (Z5, R5 and R10), after e-cig inhalation among asthmatic smokers using data from Europe between 2018–2020 [31]. This systematic analysis used data from n= 10 to 408 participants (mean age ranging from 22.6 – 58 years) with most of the studies (16/18) assessing lung function after about 5 min to 1 month of e-cig use. The other two studies examined lung function measures after 1-3 months of e-cigarette use (exposure 1–24 months). The nicotine concentration in the e-cigarettes used ranged from 0.8–24 mg/ml. The mean age of the subjects ranged from 22.6–58 years.

While these studies are short term in nature, we believe our data which assessed lung function at about 6 months of e-cig use adds to the current state of the science on the adverse impacts on the small airways after e-cig exposure. Further studies are needed to understand the respiratory health impacts of longer term e-cig use. Additionally, these previous studies were in adult populations, some of whom had comorbidities such as asthma or chronic obstructive pulmonary disease. Our study population was in relatively healthy adolescents and is the first study to our knowledge that documents the long-term adverse impact of e-cig use on lung function. Indeed, the systematic analysis by Song et al noted the dire need for relevant studies on e-cig use and lung function in vulnerable populations after concluding that there seemed to be no effect on pulmonary ventilation function after 3 months of e-cig use [31].

Furthermore, we demonstrate differences in nasal epithelial gene expression. When considering the lowest p values, the top four genes (*CEACAM4, MMP25, NCF1* and *NFAM1)* that were overexpressed in vapers compared to control participants were associated with inflammatory processes and/or roles in the immune system (Fig 3). For instance, *CEACAM4* is an orphan receptor of the *CEACAM* family that is associated with phagocytic function [32]. *MMP25* is a metalloprotease that regulates innate immune response through NF-κB signaling [33]. Mutations in *NCF1* is associated with chronic granulomatous disease [34] and variants of this gene are associated with an increased risk of developing autoimmune diseases [35-37] and recurrent spontaneous abortion [38]. *NFAM1* promotes pro-inflammatory cytokine production in both mouse and human monocytes and has been identified as a potential therapeutic target for treatment of autoimmune disease [39]which are characterized by abnormal inflammatory response [40].

While we could not find original research on *AL121956.6*. Three other top genes (*CAPS, DNAH7* and  *NWD1)* that were downregulated among vapers, compared to nonvapers have some significant implications based on reports in the literature. *CAPS* encodes the calcium binding protein calcyphosine which is associated with cellular proliferation and differentiation [41]. *DNAH7* is associated with ciliary dysfunction and may be important in understanding the molecular pathogenesis of Middle East respiratory syndrome coronavirus (MERS-CoV) [42] and COVID-19 infections [43]. *NWD1* has been demonstrated to modulate androgen receptor protein levels [44] and is involved in axonal growth, with high protein levels detected in the brain of a mouse model for seizure and temporal lobe epilepsy [45]. A recent study demonstrated that the knockdown of *NWD1* inhibited dendritic growth and synaptogenesis [46]. Taken together, vaping may be associated with increased risk of inflammation, decreased ciliogenesis and adverse impacts on brain activity and this is a serious public health concern among adolescents which must be urgently addressed.

Additionally, several genes that were differentially expressed when vapers are compared to nonvapers have also been reported in the literature. For instance, in a cohort of ten healthy never smokers, short term e-cig use was associated with altered transcriptomes of small airway epithelial cells and alveolar macrophages among all subjects [47]. Interestingly, two genes identified in this short term study were dysregulated in the opposite direction in our study. *NDC80* (FC: 0.56) and *PTGER3* (FC: -1.27) had differential expression in directions opposite to our current study. Differences in e-cig products, demographics of the study population, duration of exposure as well as the location of the biological sample (brushing nasal epithelial vs brushing the 10th–12th order bronchi). Further studies are needed to understand the effects of the increasing prevalence of e-cig use. Indeed, Sayed et al compared sputum and salivafrom e-cig users and nonusers and observed reductions in markers of airway inflammation among e-cig users compared to nonusers [48]. However, plasma concentrations of certain inflammatorycytokines, chemokines, and growth factors were higher among e-cig users. Hence it is possible that changes in airway inflammatory markers may be a counter-response to general inflammation caused by chronic e-cig use [48] and additional studies are warranted.

Several members of the mucin family (eg *MUC5AC, MUC12*) were differentially expressed among vapers and nonvapers. Mucins are O-glycosylated proteins that play an essential role in forming protective mucous barriers on epithelial surfaces and have been implicated in epithelial renewal and differentiation. These glycoproteins also play a role in intracellular signaling. In our study, several signaling pathways such as ‘cytokine-mediated’, ‘cell surface receptor’, ‘signal transduction’, ‘cytokine signaling in immune system’, ‘cytokine signaling in the immune system’, ‘G alpha signaling events’, ‘chemokine signaling, ‘NOD-like receptor’ and ‘B cell receptor’ signaling pathways were enriched. Another noteworthy pathway recently implicated in developmental biology is the *wnt* signaling pathway. In our study, *WNT5B, WNT3A* and *WNT4* were underexpressed in vapers compared to nonvapers. W*nt* signaling has been recently highlighted as a pathway whose dysregulation can affect lung disease development later in life [49, 50].

Pathways enriched for ciliary function involved genes such as the DNAH family. Of note, of the 12 members of the DNAH family of genes that were differentially expressed in our data set, 11 were underexpressed in vapers. *DNAH17 was the only gene that was overexpressed.* Most of the 11 DNAH genes have been detected in the lung and have been implicated in ciliary dyskinesia in airway epithelial cells [51] and DNAH17 is mostly expressed in the testis and are less abundant in the lung [52]. Since e-cig use is associated with significant inflammation in the lungs, albeit the dysregulation in the immune landscape differs from that of tobacco use, it is important for future studies to focus on e-cig use and its impact on systemic function as well [53].

An interesting pathway in our enrichment analysis was the inflammasome pathway. *IL1B* was upregulated in vapers (FC: 2.1) and this has been suggested that inflammasomes are activated, and similar to Lee et al, *CXCL1, CXCL2* and *NOD2* were upregulated in vapers [53]. Inflammasomes (such as nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing (NLRP)) are large protein structures primarily located in macrophages that respond to inflammatory signals [53]. Upon activation, inflammasomes cleave pro-IL1B and pro-*IL18* into *IL1B* and *IL18*, to signal the presence of xenobiotics and initiate the inflammatory response [54]. Several members of the NLRP family were differentially expressed when vapers were compared to controls (*NLRP1, NLRP3, NLRP6, NLRP12,* and *NLRP14*). All except *NLRP14* were upregulated. The dysregulation of inflammasome pathway may be an important step in immune dysregulation during chronic e-cig use. For instance, an inflammatory cytokine such as *IL1B* (upregulated in vapers) can stimulate the production of other inflammatory cytokines (eg *IL6* and *TNF*) which were also upregulated in vapers vs nonvapers in our study. Future studies may need to assess the types of e-cig devices that may stimulate different pathways due to chronic e-cig use. The study by Hickman et al highlights the importance of assessing device type. Data from third- and fourth-generation e-cig users and found an overall suppression of host defense associated with fourth-generation e-cig use [55]. This may be due to new/emerging formulations in the e-cigs such as nicotine salts and future studies need to be designed to understand the health consequences of emerging e-cigs [56].

A pediatric pulmonary review concluded that vaping may increase risk of developing chronic lung disease [57]. The authors indicated that vaping is associated with both in vitro and in vivo airway mucociliary dysfunction, increased epithelial cell, macrophage death, as well as dysregulation of airway epithelium. Indeed, data from our current study point to these adverse respiratory impacts among adolescents who use e-cigs. Our findings are important for a number of reasons: 1) it demonstrates that vape use is associated with sub clinically relevant symptoms, 2) significant changes in airway resistance that result from vape exposure may precede the development of adverse respiratory symptoms, 3) vape exposure negatively impacts adolescent lung function during a critical stage of their lung development, and 4) changes in gene expression in inflammatory pathways and genes involved in ciliary function may be biological mechanisms that underlie the development of airflow obstruction in this at-risk population.

Limitations of our study include the small sample size, the cross-sectional nature of the study, and the lack of exposure assessment specific to e-cigarettes in this cohort. Future investigations in this at-risk adolescent population will be needed to assess the longitudinal impact of habitual vape exposure on lung function outcomes and should include a more comprehensive exposure assessment of the contents of vape smoke. Despite the increased prevalence of vaping in Latinx adolescents, this is one of the first studies to address this growing health disparity. Our preliminary data indicates that vaping is associated with impaired lung function in adolescents and extensive changes in nasal epithelial gene expression. While limited in sample size, our results adds to the currently limited knowledge on the chronic effects of vape exposures. Given the paucity of information on the effects of vaping on the airway epithelium and the high-risk youth population with access to these devices, our work suggests that further research is needed to help characterize vaping exposure and its impact on lung function, nasal epithelial gene expression, and how vaping cessation may reverse these changes.

**References**

1. Rowell, T.R. and R. Tarran, Will chronic e-cigarette use cause lung disease? *Am J Physiol Lung Cell Mol Physiol*, **2015**. 309(12): p. L1398-409.

2. National Academies of Sciences, E., Medicine, Health, D. Medicine, H. Board on Population, P. Public Health, and S. Committee on the Review of the Health Effects of Electronic Nicotine Delivery, *Public Health Consequences of E-Cigarettes*, in *Public Health Consequences of E-Cigarettes*, D.L. Eaton, L.Y. Kwan, and K. Stratton, Editors. 2018, National Academies Press (US) Copyright 2018 by the National Academy of Sciences. All rights reserved.: Washington (DC).

3. Walley, S.C. and B.P. Jenssen, Electronic Nicotine Delivery Systems*.* *Pediatrics*, **2015**. 136(5): p. 1018-26.

4. Douglass, B., S. Solecki, and T. Fay-Hillier, The Harmful Consequences of Vaping: A Public Health Threat*.* *J Addict Nurs*, **2020**. 31(2): p. 79-84.

5. DHHS, U.S. Department of Health & Human Services (2016). E-cigarette use among youth and young adults: A report of the surgeon general—Executive summary. U.S. Department of Health and Human Services Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. <https://e-cigarettes.surgeongeneral.gov/documents/2016_sgr_full_report_non-508.pdf>*.* **2016**.

6. Walley, S.C., K.M. Wilson, J.P. Winickoff, and J. Groner, A Public Health Crisis: Electronic Cigarettes, Vape, and JUUL*.* *Pediatrics*, **2019**. 143(6).

7. Hammig, B., P. Daniel-Dobbs, and H. Blunt-Vinti, Electronic cigarette initiation among minority youth in the United States*.* *Am J Drug Alcohol Abuse*, **2017**. 43(3): p. 306-310.

8. Cullen  KA, A.B., Gentzke  AS, Apelberg  BJ, Jamal  A, King  BA, Notes from the field: use of electronic cigarettes and any tobacco product among middle and high school students—United States, 2011-2018.  MMWR Morb Mortal Wkly Rep; 67(45):1276-1277. **2018**.

9. Miech, R., L. Johnston, P.M. O'Malley, J.G. Bachman, and M.E. Patrick, Adolescent Vaping and Nicotine Use in 2017-2018 - U.S. National Estimates*.* *N Engl J Med*, **2019**. 380(2): p. 192-193.

10. Miech, R., A.M. Leventhal, P.M. O'Malley, L.D. Johnston, and J.L. Barrington-Trimis, Failed Attempts to Quit Combustible Cigarettes and e-Cigarettes Among US Adolescents*.* *Jama*, **2022**. 327(12): p. 1179-1181.

11. Ghosh, T.S., R. Tolliver, A. Reidmohr, and M. Lynch, Youth Vaping and Associated Risk Behaviors — A Snapshot of Colorado*.* **2019**. 380(7): p. 689-690.

12. Lanza, S.T., M.A. Russell, and J.L. Braymiller, Emergence of electronic cigarette use in US adolescents and the link to traditional cigarette use*.* *Addictive behaviors*, **2017**. 67: p. 38-43.

13. Lee, J., V. Taneja, and R. Vassallo, Cigarette smoking and inflammation: cellular and molecular mechanisms*.* *J Dent Res*, **2012**. 91(2): p. 142-9.

14. Chun, L.F., F. Moazed, C.S. Calfee, M.A. Matthay, and J.E. Gotts, Pulmonary toxicity of e-cigarettes*.* *American journal of physiology. Lung cellular and molecular physiology*, **2017**. 313(2): p. L193-L206.

15. Suzuki, T., K. Tsushima, N. Kawata, T. Matsumura, Y. Matsuura, Y. Ichimura, J. Terada, S. Sakao, Y. Tada, N. Tanabe, and K. Tatsumi, Estimation using the impulse oscillation system in patients with pulmonary sarcoidosis*.* *Sarcoidosis Vasc Diffuse Lung Dis*, **2015**. 32(2): p. 144-50.

16. Miller, M.R., R. Crapo, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, P. Enright, C.P. van der Grinten, P. Gustafsson, R. Jensen, D.C. Johnson, N. MacIntyre, R. McKay, D. Navajas, O.F. Pedersen, R. Pellegrino, G. Viegi, and J. Wanger, General considerations for lung function testing*.* *Eur Respir J*, **2005**. 26(1): p. 153-61.

17. Wong, A., K. Hardaker, P. Field, J. Huvanandana, G.G. King, H. Reddel, H. Selvadurai, C. Thamrin, and P.D. Robinson, Home-based Forced Oscillation Technique Day-to-Day Variability in Pediatric Asthma*.* *Am J Respir Crit Care Med*, **2019**. 199(9): p. 1156-1160.

18. Culver, B.H., B.L. Graham, A.L. Coates, J. Wanger, C.E. Berry, P.K. Clarke, T.S. Hallstrand, J.L. Hankinson, D.A. Kaminsky, N.R. MacIntyre, M.C. McCormack, M. Rosenfeld, S. Stanojevic, D.J. Weiner, and A.T.S.C.o.P.S.f.P.F. Laboratories, Recommendations for a Standardized Pulmonary Function Report. An Official American Thoracic Society Technical Statement*.* *Am J Respir Crit Care Med*, **2017**. 196(11): p. 1463-1472.

19. Lundblad, L.K.A., R. Miletic, E. Piitulainen, and P. Wollmer, Oscillometry in Chronic Obstructive Lung Disease: In vitro and in vivo evaluation of the impulse oscillometry and tremoflo devices*.* *Sci Rep*, **2019**. 9(1): p. 11618.

20. Ward, C.M., T.H. To, and S.M. Pederson, ngsReports: a Bioconductor package for managing FastQC reports and other NGS related log files*.* *Bioinformatics*, **2020**. 36(8): p. 2587-2588.

21. He, B., R. Zhu, H. Yang, Q. Lu, W. Wang, L. Song, X. Sun, G. Zhang, S. Li, J. Yang, G. Tian, P. Bing, and J. Lang, Assessing the Impact of Data Preprocessing on Analyzing Next Generation Sequencing Data*.* *Front Bioeng Biotechnol*, **2020**. 8: p. 817.

22. Frankish, A., M. Diekhans, A.M. Ferreira, R. Johnson, I. Jungreis, J. Loveland, J.M. Mudge, C. Sisu, J. Wright, J. Armstrong, I. Barnes, A. Berry, A. Bignell, S. Carbonell Sala, J. Chrast, F. Cunningham, T. Di Domenico, S. Donaldson, I.T. Fiddes, C. Garcia Giron, J.M. Gonzalez, T. Grego, M. Hardy, T. Hourlier, T. Hunt, O.G. Izuogu, J. Lagarde, F.J. Martin, L. Martinez, S. Mohanan, P. Muir, F.C.P. Navarro, A. Parker, B. Pei, F. Pozo, M. Ruffier, B.M. Schmitt, E. Stapleton, M.M. Suner, I. Sycheva, B. Uszczynska-Ratajczak, J. Xu, A. Yates, D. Zerbino, Y. Zhang, B. Aken, J.S. Choudhary, M. Gerstein, R. Guigo, T.J.P. Hubbard, M. Kellis, B. Paten, A. Reymond, M.L. Tress, and P. Flicek, GENCODE reference annotation for the human and mouse genomes*.* *Nucleic Acids Res*, **2019**. 47(D1): p. D766-D773.

23. Dobin, A., C.A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, and T.R. Gingeras, STAR: ultrafast universal RNA-seq aligner*.* *Bioinformatics*, **2013**. 29(1): p. 15-21.

24. Law, C.W., Y. Chen, W. Shi, and G.K. Smyth, voom: Precision weights unlock linear model analysis tools for RNA-seq read counts*.* *Genome Biol*, **2014**. 15(2): p. R29.

25. Livak, K.J. and T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method*.* *Methods*, **2001**. 25(4): p. 402-8.

26. Risso, D., J. Ngai, T.P. Speed, and S. Dudoit, Normalization of RNA-seq data using factor analysis of control genes or samples*.* *Nature biotechnology*, **2014**. 32(9): p. 896-902.

27. Palamidas, A., S. Tsikrika, P.A. Katsaounou, S. Vakali, S.A. Gennimata, G. Kaltsakas, C. Gratziou, and N. Koulouris, Acute effects of short term use of ecigarettes on Airways Physiology and Respiratory Symptoms in Smokers with and without Airway Obstructive Diseases and in Healthy non smokers*.* *Tob Prev Cessat*, **2017**. 3: p. 5.

28. Lappas, A.S., A.S. Tzortzi, E.M. Konstantinidi, S.I. Teloniatis, C.K. Tzavara, S.A. Gennimata, N.G. Koulouris, and P.K. Behrakis, Short-term respiratory effects of e-cigarettes in healthy individuals and smokers with asthma*.* *Respirology*, **2018**. 23(3): p. 291-297.

29. Flouris, A.D., M.S. Chorti, K.P. Poulianiti, A.Z. Jamurtas, K. Kostikas, M.N. Tzatzarakis, A. Wallace Hayes, A.M. Tsatsakis, and Y. Koutedakis, Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function*.* *Inhal Toxicol*, **2013**. 25(2): p. 91-101.

30. Tzortzi, A., S.I. Teloniatis, G. Matiampa, G. Bakelas, V.K. Vyzikidou, C. Vardavas, P.K. Behrakis, and E. Fernandez, Passive exposure to e-cigarette emissions: Immediate respiratory effects*.* *Tob Prev Cessat*, **2018**. 4: p. 18.

31. Song, Y., X. Li, C. Li, S. Xu, Y. Liu, and X. Wu, What Are the Effects of Electronic Cigarettes on Lung Function Compared to Non-Electronic Cigarettes? A Systematic Analysis*.* *Int J Public Health*, **2022**. 67: p. 1604989.

32. Delgado Tascón, J., J. Adrian, K. Kopp, P. Scholz, M.P. Tschan, K. Kuespert, and C.R. Hauck, The granulocyte orphan receptor CEACAM4 is able to trigger phagocytosis of bacteria*.* *J Leukoc Biol*, **2015**. 97(3): p. 521-31.

33. Soria-Valles, C., A. Gutiérrez-Fernández, F.G. Osorio, D. Carrero, A.A. Ferrando, E. Colado, M.S. Fernández-García, E. Bonzon-Kulichenko, J. Vázquez, A. Fueyo, and C. López-Otín, MMP-25 Metalloprotease Regulates Innate Immune Response through NF-κB Signaling*.* *J Immunol*, **2016**. 197(1): p. 296-302.

34. Chung, A.G., M.M. Cyr, and A.K. Ellis, Newly diagnosed chronic granulomatous disease in a 44 year old male presenting with recurrent groin cellulitis and colitis*.* *Allergy Asthma Clin Immunol*, **2013**. 9(1): p. 9.

35. Meng, Y., J. Ma, C. Yao, Z. Ye, H. Ding, C. Liu, J. Li, G. Li, Y. He, J. Li, Z. Yin, L. Wu, H. Zhou, and N. Shen, The NCF1 variant p.R90H aggravates autoimmunity by facilitating the activation of plasmacytoid dendritic cells*.* *The Journal of clinical investigation*, **2022**. 132(16): p. e153619.

36. Zhong, J., A.C.Y. Yau, and R. Holmdahl, Independent and inter-dependent immunoregulatory effects of NCF1 and NOS2 in experimental autoimmune encephalomyelitis*.* *Journal of neuroinflammation*, **2020**. 17(1): p. 113-113.

37. Zhang, L., J. Wax, R. Huang, F. Petersen, and X. Yu, Meta-Analysis and Systematic Review of the Association between a Hypoactive NCF1 Variant and Various Autoimmune Diseases*.* *Antioxidants (Basel, Switzerland)*, **2022**. 11(8): p. 1589.

38. Du, M., H. Gu, Y. Li, L. Huang, M. Gao, H. Xu, H. Deng, W. Zhong, X. Liu, and X. Zhong, A missense variant in NCF1 is associated with susceptibility to unexplained recurrent spontaneous abortion*.* *Open life sciences*, **2022**. 17(1): p. 1443-1450.

39. Juchem, K.W., A.P. Gounder, J.P. Gao, E. Seccareccia, N. Yeddula, N.J. Huffmaster, A. Côté-Martin, S.E. Fogal, D. Souza, S.S. Wang, E.R.A. Glynn, I. Yung, J. Ritchie, L. Li, J. Zheng, M.L. Mbow, J. Li, and S.K. Chanda, NFAM1 Promotes Pro-Inflammatory Cytokine Production in Mouse and Human Monocytes*.* *Front Immunol*, **2021**. 12: p. 773445.

40. Duan, L., X. Rao, and K.R. Sigdel, Regulation of Inflammation in Autoimmune Disease*.* *J Immunol Res*, **2019**. 2019: p. 7403796.

41. Clément, S., J.E. Dumont, and S. Schurmans, Loss of calcyphosine gene expression in mouse and other rodents*.* *Biochem Biophys Res Commun*, **1997**. 232(2): p. 407-13.

42. Lado, S., J.P. Elbers, M. Plasil, T. Loney, P. Weidinger, J.V. Camp, J. Kolodziejek, J. Futas, D.A. Kannan, P. Orozco-terWengel, P. Horin, N. Nowotny, and P.A. Burger, Innate and Adaptive Immune Genes Associated with MERS-CoV Infection in Dromedaries*.* *Cells*, **2021**. 10(6).

43. Hu, J., C. Li, S. Wang, T. Li, and H. Zhang, Genetic variants are identified to increase risk of COVID-19 related mortality from UK Biobank data*.* *medRxiv*, **2020**.

44. Correa, R.G., M. Krajewska, C.F. Ware, M. Gerlic, and J.C. Reed, The NLR-related protein NWD1 is associated with prostate cancer and modulates androgen receptor signaling*.* *Oncotarget*, **2014**. 5(6): p. 1666-82.

45. Yang, Q., Z. Huang, Y. Luo, F. Zheng, Y. Hu, H. Liu, S. Zhu, M. He, D. Xu, Y. Li, M. Yang, Y. Yang, X. Wei, X. Gao, W. Wang, J. Ma, Y. Ma, X. Wang, and Q. Wang, Inhibition of Nwd1 activity attenuates neuronal hyperexcitability and GluN2B phosphorylation in the hippocampus*.* *EBioMedicine*, **2019**. 47: p. 470-483.

46. Wu, Y., Q. Fu, X. Huang, Y. Luo, S. Wan, M. Peng, S. Su, X. Xu, Y. Li, X. Li, D. Sun, and C. Ke, NWD1 facilitates synaptic transmission and contributes to neuropathic pain*.* *Neuropharmacology*, **2022**. 205: p. 108919.

47. Staudt, M.R., J. Salit, R.J. Kaner, C. Hollmann, and R.G. Crystal, Altered lung biology of healthy never smokers following acute inhalation of E-cigarettes*.* *Respir Res*, **2018**. 19(1): p. 78.

48. Sayed, I.M., J.A. Masso-Silva, A. Mittal, A. Patel, E. Lin, A. Moshensky, J. Shin, C.M. Bojanowski, S. Das, P. Akuthota, and L.E. Crotty Alexander, Inflammatory phenotype modulation in the respiratory tract and systemic circulation of e-cigarette users: a pilot study*.* *Am J Physiol Lung Cell Mol Physiol*, **2021**. 321(6): p. L1134-l1146.

49. Cahill, K.M., M.R. Gartia, S. Sahu, S.R. Bergeron, L.M. Heffernan, D.B. Paulsen, A.L. Penn, and A. Noël, In utero exposure to electronic-cigarette aerosols decreases lung fibrillar collagen content, increases Newtonian resistance and induces sex-specific molecular signatures in neonatal mice*.* *Toxicological Research*, **2022**. 38(2): p. 205-224.

50. Cahill, K.M., T.K. Johnson, Z. Perveen, M. Schexnayder, R. Xiao, L.M. Heffernan, I.M. Langohr, D.B. Paulsen, A.L. Penn, and A. Noël, In utero exposures to mint-flavored JUUL aerosol impair lung development and aggravate house dust mite-induced asthma in adult offspring mice*.* *Toxicology*, **2022**. 477: p. 153272.

51. Imtiaz, F., R. Allam, K. Ramzan, and M. Al-Sayed, Variation in DNAH1 may contribute to primary ciliary dyskinesia*.* *BMC Med Genet*, **2015**. 16: p. 14.

52. Whitfield, M., L. Thomas, E. Bequignon, A. Schmitt, L. Stouvenel, G. Montantin, S. Tissier, P. Duquesnoy, B. Copin, S. Chantot, F. Dastot, C. Faucon, A.L. Barbotin, A. Loyens, J.P. Siffroi, J.F. Papon, E. Escudier, S. Amselem, V. Mitchell, A. Touré, and M. Legendre, Mutations in DNAH17, Encoding a Sperm-Specific Axonemal Outer Dynein Arm Heavy Chain, Cause Isolated Male Infertility Due to Asthenozoospermia*.* *Am J Hum Genet*, **2019**. 105(1): p. 198-212.

53. Lee, A.C., J. Chakladar, W.T. Li, C. Chen, E.Y. Chang, J. Wang-Rodriguez, and W.M. Ongkeko, Tobacco, but Not Nicotine and Flavor-Less Electronic Cigarettes, Induces ACE2 and Immune Dysregulation*.* *Int J Mol Sci*, **2020**. 21(15).

54. Lu, A. and H. Wu, Structural mechanisms of inflammasome assembly*.* *Febs j*, **2015**. 282(3): p. 435-44.

55. Hickman, E., A. Payton, P. Duffney, H. Wells, A.S. Ceppe, S. Brocke, A. Bailey, M.E. Rebuli, C. Robinette, B. Ring, J.E. Rager, N.E. Alexis, and I. Jaspers, Biomarkers of Airway Immune Homeostasis Differ Significantly with Generation of E-Cigarettes*.* *Am J Respir Crit Care Med*, **2022**. 206(10): p. 1248-1258.

56. Sullivan, L. and L.E. Crotty Alexander, A Problem for Generations: Impact of E-Cigarette Type on Immune Homeostasis*.* *Am J Respir Crit Care Med*, **2022**. 206(10): p. 1195-1197.

57. Kaslow, J.A., C. Rosas-Salazar, and P.E. Moore, E-cigarette and vaping product use-associated lung injury in the pediatric population: A critical review of the current literature*.* *Pediatr Pulmonol*, **2021**. 56(7): p. 1857-1867.

**Table 4. Top 15 biological processes and pathways for differentially regulated genes when adolescents who vaped in the last 6 months are compared to adolescents who did not vape (Based on Gene Ontology enrichment analyses). Pathways with low false discovery rate (FDR) values and negative Normalized Enrichment Scores (NES) represent downregulated biological processes while those with low FDR and positive NES represent upregulated biological processes.**



**Table 5. Top 15 biological processes and pathways for differentially regulated genes when adolescents who vaped in the last 6 months are compared to adolescents who did not vape (Based on Reactome pathway analyses). Pathways with low false discovery rate (FDR) values and negative Normalized Enrichment Scores (NES) represent downregulated biological processes while those with low FDR and positive NES represent upregulated biological processes. **

**Table 6. Top 15 biological processes and pathways for differentially regulated genes when adolescents who vaped in the last 6 months are compared to adolescents who did not vape (Based on KEGG pathway analyses). Pathways with low false discovery rate (FDR) values and negative Normalized Enrichment Scores (NES) represent downregulated biological processes while those with low FDR and positive NES represent upregulated biological processes.**

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