Electronic cigarette vaping triggers lipid mediated vocal fold mucosal injury

Vlasta Lungova¹ and Susan L. Thibeault¹

Vlasta Lungova
Department of Surgery, University of Wisconsin Madison
5105 WIMR, Madison, WI
lungova@surgery.wisc.edu

Susan L. Thibeault
Department of Surgery, University of Wisconsin Madison
5103 WIMR, Madison, WI
thibeault@surgery.wisc.edu
Corresponding Author: thibeault@surgery.wisc.edu

The authors have declared that no conflict of interest exists

21 **Abstract**

22 Electronic cigarettes (e-cigs) are nicotine delivery systems that have been touted
 23 as safer alternatives to smoking. A recently reported case of epiglottitis revealed a
 24 connection between vaping and swollen laryngeal and vocal fold (VF) structures that
 25 can lead to acute life-threatening airway obstruction. The clinical course and biopsy
 26 revealed direct epithelial injury and subsequent inflammatory reaction. Here we show
 27 that we were able to recapitulate this phenomenon in in vitro conditions. Exposure of
 28 engineered VF mucosae to 5% e-cig vapor extract for one week induced cellular
 29 damage in VF luminal epithelial cells, disrupting mucosal homeostasis and mucosal
 30 innate immune responses. Epithelial erosion was likely caused by the accumulation of
 31 solvents and lipid particles, most likely medium chain fatty acids, in the cytosol and
 32 intercellular spaces, which altered lipid metabolism and plasma membrane properties.
 33 In summary, vaping represents a threat to the VF mucosa health and airway protection.

Introduction

Recent cases of acute electronic cigarette (e-cig), or vaping, associated lung injuries (EVALI) opened a debate about the safety and health-related consequences of vaping. Multiple case reports have described atypical pneumonia and deaths in e-cig users (1–5) prompting intense scientific research focusing on effects of vaping on cellular functions of distal airways, where gas exchange takes place (6–8). As e-cigs are heated in the mouth, inhaled vaporized e-liquids pass through the throat past the larynx and vocal folds (VF), down into the lungs. Local droplet deposition in upper airways can have, therefore, physiological consequences and pose potential threats to oropharyngeal and VF health.

E-cigs are nicotine delivery systems have been touted as safer alternatives to conventional smoking and rapidly gained popularity, especially among young adults and high school age adolescents (9). E-cigs consist of prefilled or fillable cartridges with e-liquids that serve as reservoirs for vaping substances such as vehicle solvents, propylene glycol (PG) and vegetable glycerin (VG), mixed with different concentrations of nicotine (N) and flavors (F). When heated, these substances form an aerosol that is then inhaled. VG is thick with a natural sweet flavor, producing the clouds of vapors upon exhalation; PG is less viscous, producing greater throat stimulation and mimics the sensation of smoking. PG and VG give e-liquids their high viscosity. As a result, aerosols from these liquids are likely to adhere to exposed surfaces, such as the soft and hard tissues (10). Currently, an estimated 10 million US adults and over 3 million high school students are active e-cig users (9). Those that have no previous experience

with conventional cigarette smoking are at higher risk, as they exhibit increased susceptibility to lung damage and viral and/or bacterial infections (11).

In this study, we evaluated possible consequences of vaping on VF mucosal structure and function. The larynx and VF are involved in voice production and are, as parts of the conducting airways, directly exposed to inhaled vaporized e-liquids. Particularly vulnerable are epithelial cells that serve as first line of defense against inorganic, organic, and microbial intruders and protect the lamina propria beneath (12). So far, vaping associated cases of respiratory diseases have sudden onset symptoms that develop rapidly (1–3,6). Acute diseases associated with the larynx include acute laryngitis and epiglottitis and are usually caused by viral or bacterial infections (13). They cause swelling of laryngeal structures and can rapidly lead to life-threatening airway obstruction (13). A recently reported case of epiglottitis in an adolescent female patient revealed a connection between vaping and swollen laryngeal structures without signs of viral, bacterial or fungal infections (14). She was hospitalized twice and presented with acute respiratory distress, severe dysphagia, hoarseness and increased throat clearing. She reported uses e-cigs over 1 to 2 months with different fruit- or candy-flavored cartridges. Stroboscopic examination showed moderate swelling of the epiglottis and supraglottal structures, pink laryngeal mucosa with marked inflammation. Her biopsies from the arytenoids and soft palate and a sample of fluid from the laryngeal/epiglottic region revealed reactive squamous epithelial changes with focal erosions, abundance of cellular debris and thick mucus. Her clinical course and biopsy findings were highly suspicious for direct chemical injury and/or subsequent inflammatory reaction (14). Despite the importance of understanding of the biological

effect of vaping on laryngeal and VF mucosa, physiological consequences of e-liquid deposition and whether this phenomenon can be recapitulated in experimental *in vitro* conditions remains unknown.

The recent development of a three-dimensional (3D) model of human VF mucosa by our group has allowed us to mimic *in vivo* remodeling of the VF mucosa in tobacco-related diseases (15). Upon exposure to 5% cigarette smoke extract (CSE) for 1 week, we were able to induce keratotic changes and mucosal inflammation in engineered VF mucosae, composed of human induced pluripotent stem cell (hiPSC) derived VF epithelial cells and primary human VF fibroblasts dispersed in the collagen gel that mimicked the lamina propria. Here we demonstrate that exposure to 5% e-cig vapor extract (ECVE) for 1 week induces VF epithelial injury which compromises the integrity of apical cell layers, membrane-anchored luminal mucin production and clearance, and dysregulates VF mucosal immune responses. We further show that epithelial erosion is likely caused by the accumulation of vaporized lipophilic solvents and lipid particles, most probably medium-chain triglycerides, in the cytosol that alter the lipid metabolism and restructure plasma membrane properties.

Collectively, our experimental findings revealed that exposure of VF mucosa to vaporized e-liquids disrupts VF mucosal homeostasis and innate barrier functions, which represents a potential threat to the VF mucosa health, voicing and mainly airway protection, whereby raising concerns over the safety of e-cig use to other vital and essential portions of the upper airway.

Results

Histological alterations of VF mucosae exposed to 5% ECVE

To investigate the effect of ECVE on the human VF mucosa homeostasis, we utilized the recently developed hiPSC-derived model of human VF mucosa (15). HiPS cells were first differentiated into VF basal epithelial progenitors for 10 days and then reseeded on the top of collagen-fibroblast constructs and were let to differentiate for additional 22 days. At day 32, engineered VF mucosae were exposed to 5% ECVE for 1 week to mimic exposure of cells to vaping (Figure 1A) (for detailed protocol see the Material and Methods). VF mucosae treated with plain culture medium were used as negative controls. For experimental groups, we tested three different types of e-cig vapor extracts including vehicle controls with polypropylene glycol and vegetable glycerin (PG/VG) only, e-cigs with PG/VG and nicotine (PG/VG+N) and e-cigs with PG/VG+N and flavor (PG/VG+N+F), the most popular types of e-cigs. At Day 39, human engineered VF mucosae were collected for analysis.

First, we investigated alternations in morphology of VF mucosae in control and experimental groups. Histological assessment of the VF mucosae showed a typical stratified squamous architecture for all conditions (Figure 1B-E). Epithelial cells were arranged in a basal cell layer and suprabasal cell layers that flattened apically (Fig. 1B-E). Gross histological examination revealed that ECVE exposed VF mucosae appeared thinner than control (Figure 1B-E). Next, we performed Oil Red O staining on frozen unfixed sections and found red oil droplets on the surface of the VF epithelium in all ECVE exposed VF mucosae (Figure 1F-I), suggesting that e-liquids contained lipid components. When heated, e-liquids produced aerosol mixing with the culture medium,

Figure 1: Experimental design and morphology of hiPSC-derived VF mucosae exposed to

5% ECVE. (A) Schematic illustration of the experimental design. hiPS cells were first differentiated into VF epithelial cells (VF EC) for 32 days and then exposed to 5% ECVE for 1 week. We tested three different conditions – e-cigs, vehicle controls, with PG/VG only, e-cigs with PG/VG and nicotine (PG/VG+N) and e-cigs with PG/VG with nicotine and flavor (PG/VG+N+F). Control VF mucosae treated with plain culture medium were used as negative controls. At Day 39, VF mucosae were collected and analyzed by immunohistochemistry and quantitative polymerase chain reaction. (B-E) Morphology of VF mucosae in control group (B) and 5% ECVE exposed groups (C-E) showing stratified squamous VF epithelium. (F-I) Oil Red O stain on frozen unfixed sections of VF mucosae in the control group (F) and 5% ECVE exposed groups (G-I). All 5% ECVE treated samples contained lipid droplets that adhered to cell surfaces. Scale bar = 100 μm (B-E) and 50μm (F-I). Abbreviations: e-cigs, electronic cigarettes; ECVE, electronic cigarette vapor extract; hiPS, human induced pluripotent stem cells; VF, vocal folds; VFEC, vocal fold epithelial cells.

and depositing lipid droplets that adhered to cell surfaces. In order to examine whether the exposure of cells to ECVE could alter the structure and function of the VF epithelial barrier we evaluated expression of stratified epithelial markers and tested functionality of VF epithelial cells by assessing mucin and inflammatory cytokine/chemokine expression.

ECVE affects compactness of apical epithelial cell layers

We have previously demonstrated that exposure of engineered VF mucosae to 5% cigarette smoke extract (CSE) leads to VF mucosa remodeling affecting predominantly the basal epithelial cell layer with downregulation of cytokeratin (K) 14 which pathologically accumulates in the luminal cells along with K13 (15). Therefore, we sought to determine whether 1-week exposure to 5% ECVE could also affect cytokeratin production and structure of the basal cellular compartment. We first evaluated K14 and Laminin alfa 5 (LAMA5) expression and co-stained with p63. We found that K14 did not change in 5% ECVE treated groups versus controls (Figure 2A-D). Similarly, LAMA5, a marker of the basement membrane, was detected in both control and 5% ECVE treated groups (Figure 2E-H) along with p63+, a marker of basal cells (Figure 2 A-H). However, there was a reduced expression of suprabasal K13 in ECVE exposed groups, as compared to controls suggesting that apical epithelial surfaces are compromised by ECVE (Figure 2I-L). To assess the compactness of the epithelial barrier, we stained for E-Cadherin (E-Cad), a marker of cell adherent junctions. We found that in control samples, E-Cad was strongly expressed in all epithelial cell layers (Figure 2M) while in 5% ECVE exposed groups E-Cad signal was

Figure 2: Distribution and expression levels of VF structural epithelial genes. (A-D) Anti-Cytokeratin K14 (green) and anti-p63 staining (red) in control (A) and 5% ECVE exposed VF mucosae (B-D). (E-H) Anti-Laminin alpha 5 (green) co-stained with anti-p63 (red) in control (E) and 5% ECVE treated VF mucosae (F-H). (I-L) Anti-cytokeratin K13 (in green) co-stained with anti-p63 (in red) in control (I) and 5% ECVE exposed VF mucosae (J-L). (M-P) Anti-E-cadherin staining (in green) in control (M) and 5% ECVE exposed VF mucosae (N-P). Bracketed regions in panels M, N, O and P are magnified in the boxes on the right. White arrow heads point to apical cells with decreased expression of E-Cadherin. Scale bars = 100μm. (Q-T) Transcript levels of cytokeratin K14 (Q), p63 (R), K13 (S) and CDH1 (T) in control and 5% ECVE exposed VF mucosae. Error bars represent ± standard error of the mean obtained from two biological and three technical replicates. One-Way ANOVA of variance for independent or correlated samples statistical analysis along with Tukey HSD test were used to confirm statistical significance in gene expression [p-Value ≤ 0.05 (*) and p-Value ≤ 0.01 (**)].

absent in some cells in the apical cell layers (Figure 2N-P). Histological data were supported by qPCR (Figure 2Q-T). As expected, transcript levels of K14 did not change in the PG/VG+N+F group or were upregulated in the PG/VG and PG/VG+N groups as compared to control (Figure 2Q). p63 remained the same in PG/VG and PG/VG+N groups and significantly decreased in PG/VG+N+F (Figure 2R). On the other hand, unlike in control, expression levels of K13 significantly dropped down in ECVE exposed groups (Figure 2S). A similar pattern was observed in E-Cadherin (encoded by CDH1 gene), which significantly decreased in all experimental groups compared to controls (Figure 2T). These data indicate that exposure of the VF epithelium to 5% ECVE likely impairs the structure and integrity of the luminal epithelial cell layers that come into the direct contact with aerosol and toxic substances found in ECVE. Moreover, the decreased expression of p63 in the PG/VG+N+F group suggests that the damage of the luminal layers can also ultimately cause changes in basal cells.

ECVE alters membrane-associated mucin and cytokine expression

We further investigated whether exposure to 5% ECVE can alter the function of the VF epithelial protective barrier. We have previously shown that the VF epithelium is an essential mechanism of VF defense (12) which is achieved by the compact physical epithelial barrier, mucus production and secretion of cytokines/chemokines that are parts of the innate immunity. Above, we have shown that 5% ECVE exposure affects cell adherent junctions and K13 expression in the luminal cell layers. In this section, we will primarily focus on the mucin and cytokine/chemokine expression. We evaluated expression of Mucin1 (MUC1) and 4 (MUC4) which are typical membrane-associated

Figure 3: Distribution and expression levels of functional VF epithelial genes. (A-D) Red immunofluorescent staining of Mucin 1 (MUC1) in control (A) and 5% ECVE exposed VF mucosae (B-D). Bracketed regions in panels A-D are magnified on the right respectively. (E-H) Red immunofluorescent staining of Mucin 4 (MUC4) in control (E) and 5% ECVE treated VF mucosae (F-H). Scale bar = 100µm. (I) Transcript levels of MUC 1 and MUC 4 and selected genes involved in the VF immune response - interleukin IL6, IL8 and matrix metalloproteinase 2, MMP2. Error bars represent ± standard error of the mean obtained from two biological and three technical replicates. One-Way ANOVA of variance for independent or correlated samples statistical analysis along with Tukey HSD test were used to confirm statistical significance in gene expression [p-Value ≤ 0.05 (*) and p-Value ≤ 0.01 (**)].

mucins present in the human VF (12,15). Secretory proteins, such as MUC5B or MUC5C, are not produced by stratified VF epithelial cells that cover the membranous portion of the true VF (12). Our histological data confirmed that in control VF mucosae MUC1 was detected in the apical cell layer and formed a thin protective coat (Figure 3A) as previously shown in the human native VF mucosae (15). In 5% ECVE treated groups PG/VG and PG/VG+N, MUC1 expression appears to be upregulated and moved into deeper epithelial cell layers (Figure 3B, C). Notably, in the PG/VG+N+F group, the MUC1 layer remained thin, but we observed mucus clots on the epithelial surface that wrapped cell debris (Figure 3D). As for MUC4, we did not observe any significant changes in the expression pattern between control and ECVE exposed groups (Figure 3E-H). We further confirmed expression of mucins by qPCR. In PG/VG and PG/VG+N, MUC1 transcript levels were upregulated, while in PG/VG+N+F group the expression levels of MUC1 were similar to controls (Figure 3I). On the other hand, transcript levels for MUC4 decreased particularly in the PG/VG+N and PG/VG+N+F exposed VF mucosae (Figure 3I). These findings show that exposure to ECVE leads to the increased expression of luminal MUC1, but not MUC4, and formation of mucus clots that can accumulate in the laryngeal/airway lumen and impair mucus clearance.

Next, we assessed whether structural and functional changes in the VF epithelium were capable of inducing expression of cytokines and activate a VF mucosal immune response (Figure 3I). Although we found upregulation of interleukins (IL) IL6 and IL8 in ECVE exposed groups, particularly in PG/VG and PG/VG+N groups in comparison to controls, expression levels varied and were lower than previously reported in 5% CSE exposed VF mucosae (15). Moreover, MMP2 expression did not

change in control versus 5% ECVE exposed groups (Figure. 3I). We sought to further elucidate, whether this moderate inflammatory response involves selected cytokines only or whether suppression of the mucosal immune response is more general affecting cell-cell signaling, chemotaxis and rapid response of cells to infectious agents.

ECVE stimulates production of chemokines implicated in the recruitment of eosinophils

To better understand the immunomodulatory consequences of ECVE exposure, we next evaluated VF mucosa cytokine and chemokine profiles from control versus ECVE exposed VF mucosae. We performed SYBR green-based quantitative real-time RT² PCR profiling array looking at genes involved in Human Cytokine and Chemokine Expression. We used 384-well profiler plates that were designed to screen all four samples on one plate (a 4 x 96-well format). Samples were run in a triplicate (n=3; 12 samples total). For each sample, the 384-well plate contained primers for 84 genes involved in human cytokine and chemokine expression, 5 housekeeping genes and 3 negative control wells (Supplemental Table S1). CT values, fold-regulation and p-Values for all tested genes are shown in Supplemental Tables S2 and S3. Gene expression levels were normalized to reference (housekeeping) genes and control samples. Positive fold-regulation values indicate upregulated genes and are equivalent to the fold change. While negative values indicate downregulated genes and are negative inverse of the fold change (Supplemental Table S3). Genes with a fold change ≥ 2 and p-Value ≤ 0.05 were considered as significantly differentially expressed genes.

Figure 4: RT² PCR profiling analysis focusing on human cytokine and chemokine expression. (A-C) Significantly differentially expressed genes involved in mucosal inflammation in control versus PG/VG group (A), control versus PG/VG and nicotine (B) and control versus PG/VG and nicotine and flavor (C). Upregulated genes with positive fold-regulation values are highlighted in yellow color. Downregulated genes with negative fold-regulation values are highlighted in blue. The fold-change threshold was set to 2. P-values were calculated based on a Student's t-test of the replicate 2^{-ΔΔCT} values for each gene in the control group and treatment groups, and p-values less than 0.05 were considered as significant. P-value calculation used was based on parametric, unpaired, two-sample equal variance, two-tailed distribution. Samples were run in a triplicate (n=3, 12 samples total).

Our results revealed that exposure of the VF mucosae to 5% ECVE dysregulated inflammatory responses in epithelial cells and VF fibroblasts (Figure 4). In control versus PG/VG groups, we found significant modulation in 7 genes (Figure 4A), with 2 upregulated genes *BMP4* (2.89-fold) and *BMP7* (2.49-fold), and 5 downregulated genes *SPP1* (-3.18-fold), *IL23A* (-8.35-fold), *IL21* (-7.39-fold), *CXCL12* (-3.83-fold) and *CCL7* (-3.88-fold). In the PG/VG+N group we found 5 genes significantly different from controls (Figure 4B) with 1 gene upregulated *CCL11* (2.93-fold) and 4 suppressed genes including *THPO* (-2.54-fold), *IL4* (-3.77-fold), *IL23A* (-3.98-fold) and *CSF3* (-2.94-fold). When we compared the PG/VG+N+F group with the controls, 12 genes showed significant differential expression (Figure 4C); *IL6* (3.01-fold) and *CCL11* (2.97-fold) were upregulated and *SPP1* (-7.17-fold), *MSTN* (-2.59-fold), *IL4* (-4.10-fold), *IL23A* (-9.10-fold), *IL21* (-11.12-fold), *CX3CL1* (-2.89-fold), *CSF3* (-2.76-fold), *CCL5* (-4.87-fold), *BMP4* (-2.22-fold) and *ADIPOQ* (-4.34-fold) were downregulated. These data suggest that the combination of PG/VG+N+F dysregulates VF mucosal cytokine production more than with PG/VG and PG/VG+N. Suppression of chemokines, such as *CCL5*, *CCL7*, *CX3CL1*, *IL23A*, *IL21* and *CSF3*, may cause the delay in mucosal VF responses to pathogens (7), as these chemokines are implicated in recruitment of monocytes/macrophages and neutrophils to the site of inflammation (16,17). Suppression of cytokines with anti-inflammatory cytoprotective function, such as *IL4* (18) may slow down the VF mucosal repair and regeneration. On the other hand, consistent elevated transcript levels of *CCL11* indicate that ECVE can activate migration of eosinophils that are otherwise involved in allergic reactions (19). The differences in the secretion of cytokines/chemokines suggest that ECVE likely impairs innate epithelial

barrier function along with VF fibroblast immune responses, which can lead to the development of allergic inflammation and/or decreased protection and recovery time of the VF epithelial barrier.

ECVE exposure causes imbalance in lipid cytosolic content and alters plasma membrane properties.

We further investigated whether altered VF epithelial structure and function can be associated with oil droplet deposition and examined the ultrastructure of VF epithelial cells with transmission electron microscope (TEM). In ECVE exposed VF mucosae, but not in controls, we found numerous of tiny dark spots (more than likely droplets) along with large white lipid aggregates, inside the cytoplasm and intercellular spaces (Figure 5A-H). The presence of inclusions between plasma membranes dilated the space between neighboring cells and impaired cell junctions (Figure 5C). Moreover, in the PG/VG +N and PG/VG+N+F groups, outermost epithelial cells detached from the underlying cell layers (Figure 5E, G), some exfoliated cells became loaded with lipid particles and dark pigments (Figure 5G, H) and resembled lipid laden macrophages (6,7). The origin of the cytoplasmic lipid aggregates and/or dark pigments is not clear. Since VF epithelial cells are not active producers of lipid components, such as pulmonary surfactants (7), we speculate that these are from exogenous sources. Despite the fact that the VF epithelial cells are not active lipid producers they possess the enzymatic machinery involved in lipid/fatty acid metabolism to maintain phospholipid by-layers and vital cellular functions. To investigate whether the cytoplasmic accumulation of lipid droplets can affect VF mucosal cell metabolic activity related to

Figure 5: Transmission electron microscopy of the apical region of the VF epithelium. (A, B) Ultrastructure of VF epithelial cells of the control VF mucosa. (C, D) Ultrastructure of VF epithelial cells exposed to PG/VG only. Cytosol of the epithelial cells contains dark droplets and/or pigments. White solid arrows denote white aggregates that accumulate in the cytosol and intercellular space. (E, F) Ultrastructure of the VF epithelial cells exposed to PG/VG and nicotine. White solid arrows point to white lipid aggregates in the cell cytoplasm. Apical cells tend to detach from the underlying epithelial cell layers (G, H) Ultrastructure of the VF epithelial cells exposed to PG/VG and nicotine and flavor. White solid arrows point to white lipid aggregates and dark droplets in the cell cytoplasm of detached cells. Some exfoliated cells became loaded with lipid particles and dark pigments and resembled lipid laden macrophages.

fatty acid/glycerol breakdown and/or recycling we performed SYBR green-based quantitative real-time RT² PCR profiling array looking at genes involved in Human Fatty Acid Metabolism. As described above, a 4 x 96-well format (384-well plates) was used to screen all four samples on one plate and data were run on three separate plates (n=3, 12 samples total). For each sample, the 384-well plate contained primers for 84 genes involved in human fatty acid metabolism, 5 housekeeping genes and 3 negative control wells (Supplemental Table S4). CT values, fold-regulation values and p-Values for all tested genes are included in the Supplemental Tables S5 and S6. As mentioned above, positive fold-regulation values indicate upregulated genes and are equivalent to the fold change, while negative fold regulation values indicate downregulated genes and are negative inverse of the fold-change (Supplemental Table S6). Genes with a fold change ≥ 2 and p-Value ≤ 0.05 were considered as significantly differentially expressed genes.

Our results show that the PG/VG group was at least affected by 5% ECVE as only 2 genes were significantly different from controls. ACAA1 (-2.01-fold) and ACSL5 (-4.49-fold) (Figure 6A-C) were significantly downregulated (Figure 6B). In other experimental groups, PG/VG+N and PG/VG+N+F, the effect of ECVE was more obvious (Figures 7 and 8). We found 9 and 5 significantly differentially expressed genes in PG/VG+N and PG/VG+N+F, respectively. Upregulated genes included ACADM with a fold change of 32.85 in PG/VG+N and 9.59 in PG/VG+N+F (Figures 7 and 8). This gene encodes the enzyme, Acyl-Coenzyme A dehydrogenase, that works on medium-chain fatty acids and is involved in medium-chain fatty acid degradation in mitochondria (20). On the other hand, downregulated genes, ACSBG2 (-5.38-fold), ACSL5 (-3.12-

Figure 6: RT² PCR profiling analysis focusing on human fatty acid metabolism in control and PG/VG exposed VF mucosae. (A) Volcano Plot identifies significant gene expression changes by plotting the log2 of the fold changes in gene expression on the x-axis versus their statistical significance on the y-axis. The center vertical line indicates unchanged gene expression, while the two outer vertical lines indicate the selected fold regulation threshold: fold-change ≥ 2 . The horizontal line indicates the selected p-value threshold ≤ 0.05 . (B) A table showing the list of differentially expressed genes in control versus PG/VG test group with fold-regulation values and p-values. P-Value highlighted in red color indicate significantly differentially expressed genes with fold change greater than 2 and a p-Value less than 0.05. (C) Graph showing differentially expressed genes in control versus PG/VG test group with fold-regulation plotted on the x-axis. Downregulated genes are highlighted in blue.

fold, ACSM5 (-3.62-fold), CPT1B (-2.98-fold) in PG/VG+N group and ACSBG2 (-3.58-fold), ACSM5 (-3.07-fold) in PG/VG+N+F group (Figures 7 and 8) encode enzymes needed for the synthetic reaction of fatty acids with acyl-Coenzyme A and adenosine triphosphate in the cytosol which is required for fatty acid activation and their translocation into mitochondria (21,22). Notably, significantly downregulated ACSM5 in PG/VG+N and PG/VG+N+F and other downregulated members of the family, such as

Figure 7: RT² PCR profiling analysis focusing on human fatty acid metabolism in control and PG/VG and nicotine exposed VF mucosae. (A) Volcano Plot identifies significant gene expression changes by plotting the log2 of the fold changes in gene expression on the x-axis versus their statistical significance on the y-axis. The center vertical line indicates unchanged gene expression, while the two outer vertical lines indicate the selected fold regulation threshold: fold-change ≥ 2 . The horizontal line indicates the selected p-value threshold ≤ 0.05 . (B) Table showing the list of differentially expressed genes in control versus PG/VG and nicotine test group with fold-regulation values and p-values. P-Values highlighted in red color indicate significantly differentially expressed genes with fold change greater than 2 and a p-Value less than 0.05. (C) Graph showing differentially expressed genes in control and PG/VG and nicotine test group with fold-regulation plotted on the x-axis. Upregulated genes are highlighted in yellow, downregulated genes are highlighted in blue.

Figure 8: RT² PCR profiling analysis focusing on human fatty acid metabolism in control and PG/VG and nicotine and flavor exposed VF mucosae. (A) Volcano Plot identifies significant gene expression changes by plotting the log2 of the fold changes in gene expression

on the x-axis versus their statistical significance on the y-axis. The center vertical line indicates unchanged gene expression, while the two outer vertical lines indicate the selected fold regulation threshold: fold-change ≥ 2 . The horizontal line indicates the selected p-value threshold ≤ 0.05 . (B) A table showing the list of significantly expressed genes in control and PG/VG and nicotine and flavor test group with fold-regulation values and p-values. P-Values highlighted in red color indicate significantly differentially expressed genes with fold change greater than 2 and a p-Value less than 0.05. (C) Graph showing differentially expressed genes in control and PG/VG and nicotine and flavor test group with fold-regulation plotted on the x-axis. Upregulated genes are highlighted in yellow, downregulated genes are highlighted in blue.

Overall, these findings show that the inefficient clearance of lipid and solvent substances in the cytosol dysregulates the lipid metabolism and plasma membrane properties. Besides in cytoplasm, lipid particles also deposit in the intercellular spaces impairing the integrity of the epithelial barrier with the secondary effect on communication between neighboring cells and cell-immune system signal transduction.

Discussion

Our recently developed model of human VF mucosa holds great promise for studying toxicity and mechanisms of vaping-related cellular injuries in human VF stratified epithelium. Here we show that exposure of cells to 5% ECVE for one week was sufficient to induce cellular damage in VF apical epithelial cells, which disrupted the VF mucosal homeostasis and innate barrier function. Our results correlate with clinical observations (14), as well as results published for other types of epithelia, most notably, airway and nasal epithelia (7,25–27), suggesting that responses of cells to ECVE could be both, universal and tissue specific. Commonality with other studies includes compromised mucociliary clearance, immune responsiveness and aberrant lipid homeostasis. Among e-cig users, a significant increase in membrane-anchored mucins, and an increase in the ratio of secretory mucins MUC5AC to MUC5B has been reported compared to non-smoking participants (25). Nasal scrapes from e-cig users have shown significantly decreased expression of early growth response markers essential for the host-defense mechanisms as compared to cigarette smokers (26). Histopathological examination of lung biopsies and bronchoalveolar lavage (BAL) obtained from patients with EVALI revealed pigmented lipid laden macrophages and cell debris in the BAL fluid samples (5,6,27). In animal studies, mice receiving e-cig vapor exposure for 4 months failed to develop pulmonary inflammation and emphysema and exhibited delayed but enhanced response to the infectious agents (7). Moreover, suppression of the immune system was accompanied with altered lipid homeostasis both in resident alveolar macrophages and epithelial cells that secrete pulmonary surfactants (7). Specific to laryngeal and VF tissue, in a rat model, exposure to e-cig

vapors for 4 weeks caused mild squamous metaplasia and hyperplasia and moderate subepithelial inflammation (24). However, differences between control and experimental animals were not statistically significant. Moreover, histological VF transversal sections of e-cig exposed VF exhibited cell debris that accumulated in the laryngeal lumen. These findings correspond with our observations.

In this study, comprehensive genomic and structural analyses of human VF mucosal cells show that exposure of cells to 5% ECVE disrupts the apico-basal polarity of the VF stratified squamous epithelium. Under healthy steady-state conditions, the basal cellular compartment firmly anchors the VF epithelium to the LP and provides the reserve of cells necessary for self-renewal (15,28), while apical differentiated cell layers face the external environment and perform specific functions, most notably barrier formation (12), mucus secretion (29), sensory transduction and immunological surveillance (30,31). Both domains form the compact VF epithelium held together by cell junctions that provide structural support and seal intercellular spaces (12). Induced epithelial injury, that removes apical cell layers, compromises the function of the entire epithelial protective barrier and exposes basal proliferating cells to further damage and pathogen infiltration (Figure 9). Detached cells wrapped in mucus accumulate in the airway lumen, which may contribute to increased throat clearing and coughing in e-cig users (11,14). Our findings also show moderate inflammation in VF mucosae treated with 5% ECVE, which is in accordance with published data (7,26,32). Nevertheless, we evaluated cytokine/chemokine expression in VF mucosal cells without the presence of immune cells and macrophages, which could influence their transcript levels. Further validation of our 3D *in vitro* system, and cultivation of VF mucosal cells with a population

Figure 9: Schematic illustration showing the effect of 5% ECVE on the vocal fold stratified epithelium. Vocal fold epithelium on the left represents healthy stratified squamous epithelium with the mucus protective layer that covers the luminal epithelial surface. Vocal fold epithelium on the right side represents VF epithelium exposed to 5% PG/VG and nicotine and flavor. Due to the lipid droplet deposition superficial cell layers detach from the underlying cell layers and are wrapped in mucus. Removal of apical cell layers disrupts the signaling between the VF epithelial cells and fibroblast with immune cells. We detected upregulation of genes involved in recruitment of eosinophils, but not macrophages or neutrophils, which can lead to the development of allergic inflammation and/or decreased protection and recovery time of the vocal fold epithelial barrier. Abbreviations: Lr, larynx; OC, oral cavity; Ph, pharynx; Tr, trachea, VF, vocal folds.

of immune cells, will be extremely useful to provide a profound analysis of the effect of ECVE on VF mucosal inflammation.

The proposed mechanism underlying structural and functional changes of VF epithelium is likely associated with defective lipid metabolism and excess lipid/solvent particles that accumulate in the cytosol and intercellular spaces as confirmed by Red O stain and TEM. Lipid particles can be derived from nicotine and flavorings added to e-liquid to intensify the taste and/or enhance vaping experience (8). We found significant upregulation of ACADM in the VF mucosae exposed to commercially available e-liquids with nicotine and nicotine and flavor supporting the fact that lipid aggregates found in the cell cytoplasm likely contain medium-chain fatty acids/triglycerides that pass through

the cell membrane by passive diffusion or via carriers linked to FABP proteins (23). Increased cytosolic deposition of these fatty acids is likely caused by downregulation of Acyl-CoA synthetase medium-chain family members - ACSM5, ACSM4 and ACSM3 that fail to activate fatty acids and prevent their translocation into mitochondria for final degradation (Figure 10).

Figure 10: Schematic illustration of the effect of 5% ECVE on VF epithelial lipid homeostasis. Exogenous fatty acids, that likely contain medium-chain fatty acids/triglycerides (MCFA), diffuse across the plasma membrane into the cytosol and activate ACADM gene encoding an enzyme, medium-chain acyl -CoA dehydrogenase (MCAD) in mitochondria. Simultaneously, an increased deposition of MCFA in the cytosol inhibits synthetic reactions of MCFA with acyl-CoA and adenosine triphosphate (ATP) via downregulation of ACSM5, ACSM4 and ACSM3 in the cytosol. As a result, MCFA are not translocated into mitochondria and remain in the cytoplasm. Besides the failure in MCFA activation, ECVE also likely targets fatty acid binding proteins, FABP, that facilitate transport of lipids and/or lipid soluble substances across the plasma membrane and regulate lipid cytoplasmic content. Structural changes of the transport system due to downregulation of FABPs can cause uncontrolled influx of lipid and/or solvent particles into the cells. Lipid droplets and lipophilic solvents can also deposit between the plasma membranes in the intercellular space.

Another target of ECVE could be inactivation of fatty acid binding proteins, FABPs, in the plasma membrane that assist in the fatty acid transport across the membrane and regulate fatty acid content (23). Inactivation of these proteins can lead to uncontrolled influx of fatty acids or lipophilic solvents into the cells. So far, the role of FABPs has been mostly studied in tissues with the high lipid and/or glucose metabolism such as adipose tissue, liver, intestine, heart or skeletal muscles (33–35). Several FABP isomorphs, such as FABP7, have been also found in the epidermis, brain or lungs (23,33), however, their role in these tissues is poorly understood. It has been shown that dysfunction of FABPs in skeletal and cardiac muscles causes lipotoxicity which leads to metabolic diseases (23). Pharmacological manipulations of FABP functions can, therefore, serve as promising future targets to correct lipid fluxes and regain metabolic homeostasis (33). Whether targeting *FABPs* can also prevent cellular damage in the stratified VF epithelium in response to ECVE remains to be investigated.

Collectively, our findings revealed two important aspects that deserve further attention. First, in line with previous studies (7,11) there is an urgent need to investigate the physiological effects of e-liquids currently on the market, especially now, as the market is still evolving, and new products become available. We detected significant differences in expression levels of genes involved in medium fatty acid/ triglyceride metabolism in commercially available products, with nicotine and nicotine and flavor. In general, medium-chain triglycerides represent a major risk factor associated with vaping along with vitamin E acetate (6) and belong to priority toxicants to measure in bronchoalveolar lavage fluid in patients with acute respiratory illness (6). Wide testing of

commercial products is necessary to identify their potential risks to the individual's health.

Second, vaping, undoubtedly, represents a high potential risk to voice production and airway protection, in acute setting as well as prospective long-term chronic conditions. The healthy VF are strongly dependent on the compact epithelial barrier as they undergo constant collisions during the vibratory cycle. During conversational talk, average vibratory rate of the VF ranges from 100 to 150 hertz (Hz) for males, 180 to 250 Hz for females (Hz units means number of vibratory cycles per second), which further increases during singing and may go up to 1000 Hz in soprano operatic singers (36,37). Detachment of apical cell layers burdened with lipid content in combination with constant mechanical stress can lead, ultimately, to chronic VF pathophysiological changes and life-long impairment of voice quality. Given the fact that vaping is highly popular among high school students and young adults still pursuing their professional carriers is especially alarming, as occupation-related voice disorders prevail among treatment -seeking individuals (38–40). Future clinical studies are necessary to confirm whether e-cig users are more susceptible to chronic voice disorders as compared to conventional cigarette smokers and non-smokers.

Material and Methods

Study design. The primary goal of this study was to determine the effect of vaporized e-liquids on VF mucosa remodeling and inflammation and test whether we can recapitulate clinical findings in in vitro experimental conditions. We first evaluated expression pattern of key structural and functional VF epithelial genes and then provided the comprehensive genomic analysis focusing on the expression of human chemokine and cytokines that are associated with VF mucosal immune responses and inflammation. The ECVE effects were measured relative to control VF mucosae treated with plain medium and we tested three different experimental conditions with PG/VG only, PG/VG and nicotine and PG/VG, nicotine and flavor – the most popular type of e-cig. We also aimed to further investigate the possible mechanisms responsible for the epithelial injury and provided comprehensive genomic analysis focusing on human fatty acid metabolism that identified medium-chain fatty acids as priority toxicants causing epithelial cell detachment. Overall, thirty-eight 3D constructs were generated in this study to provide at least two biological replicates for each experimental procedure. 3D constructs composed of vocal fold basal progenitors that did not attach to the collagen constructs properly were excluded from the study. A major limitation of this study was the absence of immune cells in our in vitro system, which could influence the transcript levels of chemokines and cytokines expressed by mucosal cells. CT values, fold change regulation values and p-Values for all tested genes are provided in Supplementary Materials. This in vitro system provided mechanistic insight into the effect of vaping on the VF mucosa health and function and serves as a necessary foundation for studying vaping in combination with infectious agents involved in acute epiglottitis and laryngitis.

564

565 ***Human iPS-GFP cell culture and differentiation.*** For differentiation of hiPSC-derived

566 VF epithelium we followed our recently published protocol (15). Briefly, human iPS-GFP

567 IMR-90-4 reporter cells were maintained in an undifferentiated state in mTsr1 media

568 on plates coated with Matrigel and were routinely passaged with Versene (StemCell

569 Technologies, Vancouver, CA) in a ratio of 1:6. When cells reached 80% confluency,

570 definitive endoderm induction was performed (Day 1) using RPMI medium with

571 Glutamax (Gibco, Life Technologies) supplemented with 100ng/ml Activin A (PeproTech,

572 Rocky Hill, NJ, USA), 25ng/ml Wnt 3a (R&D System, Minneapolis, MN, USA) and 10 μ M

573 Y-27632 (R&D System, Minneapolis, MN, USA) for one day and RPMI media with

574 Glutamax supplemented with 100ng/ml Activin A and 0.2% fetal bovine serum (FBS) for

575 additional two days. At Day 4, anterior foregut endoderm (AFE) differentiation was

576 performed. RPMI medium was replaced by DMEM/F12 medium with Glutamax (Gibco,

577 Life Technologies) supplemented with N2 and B27 supplements (Gibco, Life

578 Technologies), ascorbic acid 0.05 mg/ml (MiliPore Sigma, St. Louis, MO),

579 monothioglycerol (MTG) 0.4 mM (MiliPore Sigma, St. Louis, MO) (here refer as DMEM

580 basal medium), 200ng/ml Noggin (R&D System, Minneapolis, MN, USA) and 10 μ M

581 SB431542 (Tocris, Minneapolis, MN, USA) for four days. Medium was changed daily.

582 After 4 days of AFE treatment, at Day 8, AFE derived cells were differentiated into VF

583 basal progenitors (VBP) to induce expression of stratified markers for an additional 4

584 days. We used DMEM basal medium supplemented with 1% penicillin-streptomycin

585 (Invitrogen, Carlsbad, CA, USA) and a combination of FGF growth factors (FGF2

586 250ng/ml; FGF7 100ng/ml and FGF10 100ng/ml). FGFs signals were purchased from

R&D System (Minneapolis, MN, USA). Medium was changed every other day. Half-way through their differentiation, at Day 10, VFB were mildly detached with 0.05% TE trypsin in EDTA (Gibco Life Technologies) and transferred to the top of the collagen-fibroblasts constructs to create organotypic VF mucosae.

Organotypic VF mucosa cultures. One day before VBP re-seeding (Day 9), collagen constructs were prepared (15,41) by combining high-concentration rat tail collagen (4mg/ml; 80% final volume BD Biosciences) and 10xDMEM (10% final volume; Millipore Sigma, St. Louis, MO, USA) on ice and adjusting pH (between 7.2 and 7.3) with 1N NaOH. VF primary fibroblasts 21T cells, passage P5 - P6, were resuspended in ice-cold FBS (10% final volume; 500 000 cells/ml final volume) and added to a collagen mixture. A mixture of collagen gel and VF fibroblasts was plated on transwell culture inserts (Corning, Millipore Sigma, St. Louis, MO, USA), 2ml per a 6-well culture insert, and solidified for one hour in a tissue incubator at 5% CO₂, 37°C degrees. After one hour, collagen was gently detached with a pauster pipette and constructs were flooded with DMEM basal medium, returned into an incubator and left at least 24 hours to allow for gel contraction. The next day, VBP (Day 10) were mildly trypsinized and plated on collagen constructs at high density in 100 µl DMEM basal medium supplemented with high concentration of FGF2 (250ng/ml), FGF10 (100ng/ml) and FGF7 (100ng/ml). Cells were allowed to attach for at least two hours and were then were flooded with DMEM basal medium supplemented with high levels of FGFs as mentioned above and cultivated for an additional two days to complete VBP differentiation (Day 12). On Day 12, DMEM basal medium was changed for conditional flavonoid adenine dinucleotide

(FAD) medium supplemented with high levels of FGFs and VBP were further differentiated as submerged cultures for 2 days. At Day 14, conditional culture medium with FGFs was aspirated from the upper inserts and cells were cultivated at the Air/Liquid interface (A/Li). The A/Li was performed in conditional FAD medium with FGFs for the first 4 days and plain FAD medium for an additional 2 weeks. FAD medium was freshly prepared every week. It consisted of the DMEM medium and F12 in ratio 1:3 (Gibco Life Technologies), supplemented with 2.5 ml FBS, 0.4 µg/ml hydrocortisone (Millipore Sigma, St. Louis, MO, USA) 8.4ng /ml cholera toxin (Millipore Sigma, St. Louis, MO, USA), 5 µg/ml insulin (Millipore Sigma, St. Louis, MO, USA), 24µg/ml adenine (Millipore Sigma, St. Louis, MO, USA), 10ng/ml epidermal growth factor, 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA). In submerged cultures, 1 ml of FAD was applied on transwells with collagen constructs and 2 ml were applied in the basolateral chamber. FAD was changed every other day. To create the A/Li, FAD medium was placed in the basolateral chamber only and changed three times a week. Conditional FAD medium was formed by cultivation of FAD with human primary VFF 21T cells for 24hours in 37°C in 5% CO₂-humidified atmosphere. After 24hours, medium was collected, sterile-filtered and stored at -20° C. The ratio of 30:70 (30% for conditional and 70% for fresh FAD medium) was used in the experiment.

Preparation of the electronic cigarette vapor extract (ECVE). ECVE (100%) was generated as recently described (15). Briefly, e-cigarette vapors (sold by Infinite Vapor, infinitevapor.com) were bubbled through 30 ml the DMEM/F12 medium (Gibco Technologies) in a disposable 50 ml tube with the use of an experimenter-operated

syringe. Human vaping was modeled with two short puffs (2 seconds) with long delays between puffs (30 seconds). Two short puffs were repeated 15x (30 puffs total), which was equivalent to three conventional cigarettes used in our previous study (15) for each experimental condition. Aerosolized vapors that were drawn through the end of the e-cigarette during vaping, were bubbled through the DMEM medium. The obtained medium was considered 100% EVCE. To ensure standardization between experiments, ECVE was sterile-filtered through a 0.2 mm filter, aliquoted, and stored at -80°C. Before usage, ECVE was quickly thawed and diluted with the FAD medium to the indicated concentration and used the same day. We prepared three different 100% ECVE extracts using three distinct e-liquid cartridges for three different experimental conditions: PG/VG only (vehicle control), 0% nicotine (Hell Vapors; batch number: G0391); the e-liquid with PG/VG with 1.8% nicotine (Hells Vapors, batch number: C1291) and the e-liquid with PG/VG with 1.2% nicotine and Unicorn Poop flavor (Drip Star, batch number: E0891).

Functional experiments using 5% EVCE. To mimic chronic exposure of VF mucosae to ECVE, inserts (upper chamber) containing engineered VF mucosae at Day 32 of differentiation were flooded with FAD medium supplemented with 5% ECVE for 1 week. We used 5% ECVE with PG/VG only, 5% ECVE with PG/VG+N and 5% ECVE with PG/VG+N+F. The lower chamber was flooded with plain FAD medium. Engineered VF mucosae flooded with plain FAD medium were used as negative controls. Medium was changed every day in both chambers. After one week of exposure to the 5% ECVE engineered VF mucosae were characterized with immunohistochemistry (IHC) and

quantitative polymerase chain reaction (qPCR) to investigate expression levels of clinically relevant genes in VF mucosal cells. We also performed Oil Red O stain on frozen VF mucosa sections to detect lipid particles and TEM to evaluate the ultrastructure of VF apical epithelial cell layers. Thirty-eight 3D constructs were generated in this study to enable at least two biological replicates for each experimental procedure. VBP that did not attach to the collagen constructs properly were excluded from the experiment.

Cryosectioning and Oil Red O stain. At Day 39, medium was aspirated from the control and 5% ECVE exposed VF mucosae; 3D constructs were briefly washed in PBS immediately embedded in separate molds with O.C.T (Sakura, Hayward, CA) mounting medium and placed on dry ice to freeze tissues. Blocks were stored in the freezer -80°C. Before cryosectioning, blocks were removed from the freezer, allowed to warm up to -21°C in a cryostat (Leica CM3050S) and cut to 5 µm thick sections, (chamber temperature -21°C and objective temperature -21°C). Sections were collected on pre-coated slides and immediately used for Oil Red Oil staining. Oil Red staining was performed using Oil Red O Stain Kit (Lipid Stain) (Abcam, Cambridge, CA, USA) following manufacturer's protocol. Samples were contra-stained with hematoxylin and mounted with Vectashield without DAPI (Vector Laboratories; Peterborough, UK). Images were taken with a Nikon Eclipse E600 with a camera Olympus DP71, and were adjusted for brightness using the installed DP 71 software, Olympus Corporation.

Immunohistochemistry (IHC) of collagen gel constructs. At Day 39, collagen gel constructs were first washed in PBS, fixed in fresh 4% paraformaldehyde for 15 min at RT and embedded in histogel (Thermo Fisher Scientific, Waltham, MA, USA). Constructs were dehydrated in a series of ethanol, treated with xylene, embedded in paraffin, and cut to 5µm thick serial sections. Sections were then deparaffinized, rehydrated and stained using a standard IHC protocol (15). Antigen retrieval was performed by heating sections in sodium citrate pH = 6 at 80°C water bath for two hours. Primary antibodies used included rabbit anti-Laminin alfa 5 diluted at 1:100 (Abcam; Cambridge, UK), anti-rabbit cytokeratin K14 diluted to 1:250 (Proteintech, Rosemont, IL, USA), anti-rabbit cytokeratin K13 diluted to 1:100 (Abcam, Cambridge, CA, USA), anti-rabbit E-Cadherin diluted to 1:250 (Cell Signaling, Danvers, MA, USA), anti-mouse p63 diluted to 1:100 (Santa Cruz Biotechnology, Dallas, TX, USA), and anti-mouse MUC1 and MUC4 diluted to 1:200 (both Abcam, Cambridge, CA, USA). Secondary antibodies used were Alexa Fluor TM 488 goat anti-rabbit (Invitrogen, Carlsbad, CA, USA) at the dilution 1:500, Cy3-cojugated goat anti-mouse at the dilution 1:200 (both Jackson ImmunoResearch; West Grove, PA, USA). They were applied 1h and 30 min at RT. Slides were mounted using Vectashield with DAPI (Vector Laboratories; Peterborough, UK). Images were taken with a Nikon Eclipse E600 with a camera Olympus DP71, and were adjusted for brightness using the installed DP 71 software, Olympus Corporation.

Transmission electron microscopy. For transmission electron microscopy, gels were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in a 0.1M sodium cacodylate

buffer (pH 7.4) overnight at 4°C and processed using routine techniques. Briefly, gels were washed in a 0.1M sodium cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature. Tissues were dehydrated in graded ethanol series, rinsed twice in propylene oxide, and embedded in Epon 812 epoxy resin (Polysciences, Inc., Warrington, PA) under vacuum. Finally, the samples were flat embedded between glass slides. After resin polymerization, one of the two glass slides were removed, and blank resin cylinders were glued to the sections. The gels were thin sectioned for TEM using a Leica EM UC6 Ultramicrotome and stained with Reynolds lead citrate and 8% uranyl acetate in 50% EtOH to increase contrast. The sections were viewed with a Philips CM120 electron microscope and images were captured with a MegaView III side mounted digital camera.

Isolation of VF mucosal cells for qPCR. To isolate populations of VF mucosal cells from the whole constructs, collagen gel was first dissolved using collagenase (Gibco™ Collagenase, Type I, Powder; 17018029 Gibco™). Briefly, old medium was aspirated, and constructs were washed twice in PBS. Collagenase type I at working concentration 100U/ml was added to the upper (1ml) and lower chambers (2ml), with VF mucosa being fully submerged. Constructs were incubated at 37°C for at least 2 – 3 hours, or until the collagen completely dissolved and cells got loose. The cell suspension was then transferred into a 15ml conical tube. Cells were centrifuged for 5 min at 10,000 rpm, washed and resuspended in PBS and transferred into a 1.5ml tube. Cells were again centrifuged for 5 min at 10,000 rpm, the supernatant was aspirated, and cell pellet was stored at -80°C.

RNA isolation and qPCR. Cells isolated from the whole constructs were used for RNA isolation using ReliaPrep™ RNACell Miniprep System (Promega, Madison, WI) following manufacturer's protocol. One thousand ng of RNA was reverse transcribed to cDNA using reverse transcription reagents (Go Script, Promega, Madison, WI) per manufacturer's protocol. Total volume of 0.4µl of cDNA was used per 20µl real time qPCR reaction using Power Up Sybr Green Master Mix (Applied Biosystem, Foster City, CA, USA) and run for 40 cycles in triplicates on a 7500 Fast Real Time PCR System machine (Applied Biosystem, Foster City, CA, USA), according to manufacturer's instructions. Gene specific primers are listed in Table 1. Relative gene expression, normalized to beta-Actin (Delta CT), and control VF mucosae (Delta Delta CT), was calculated as fold change using the $2^{(-\Delta\Delta CT)}$ method. If undetected, a cycle number 40 was assigned to allow fold change calculations. Data are presented as the average of the two biological and three technical replicates $\pm$ standard error of the mean. One-Way ANOVA of Variance for Independent or Correlated Samples analysis along with Tukey HSD test were used to confirm statistical significance in gene expression [$p \leq 0.05$ (*) and $p \leq 0.01$ (**)].

Gene	Forward (5'-3')	Reverse (5'-3')
Tp63	CACCATGTGAGCTCTTCCTATC	AGGTAGCCTCTTACTTCTCCTT
Cytokeratin K14	AGTCCCTACTTCAAGACCATTGAG	GGTTCAACTCTGTCTCATACTTGG
Cytokeratin K13	TCAAGACACGTCTGGAGCAG	AAGTCAGACAGTGAGGGGTCT
Beta-Actin	ACGTTGCTATCCAGGCTGTGCTAT	CTCGGTGAGGATCTTCATGAGGTAGT
CDH1	CGATTAAAGGTGGAGAGAGGACTG	AATGAATGGTGGACAGACACAGG
MUC1	AGACGTCAGCGTGAGTGATG	GACAGCCAAGGCAATGAGAT
MUC4	GGGAAGAAAGGCCCAACTAC	CTATGCTGACGGGTGGAAT
IL6	AAGCCAGAGCTGTGCAGATGAGTA	GCTGCGCAGAATGAGATGAGTTGT
IL8	AGACATACTCCAAACCTTTCCACCC	TCCAGACAGAGCTCTCTTCCATCA
MMP2	AGAAGGATGGCAAGTACGGCT	AGTGGTGCAGCTGTCATAGGATGT

Table 1: Genes and primers used in the study

RT2 PCR profiling. Cells isolated from whole constructs were used for RNA isolation using ReliaPrep™ RNACell Miniprep System (Promega, Madison, WI) following manufacturer's protocol, Five hundred ng of RNA was reverse transcribed to cDNA using a reverse transcription RT² First Strand Kit (Qiagen, Hilden, Germany) that is recommended to use in combination with RT² Profiler PCR Arrays and we followed manufacturer's protocol. Immediately after cDNA reverse transcription we followed with RT² Profiler PCR Arrays. The cDNA was first diluted with nuclease-free water and then added to the RT² SYBR Green ROX™ qPCR Mastermix (Qiagen, Hilden, Germany) according to manufacturer's protocol. We used RT² Profiler PCR Arrays for Human Cytokines and Chemokines (PAHS-150Z), and Fatty Acid Metabolism (PAHS-007Z), both purchased from Qiagen (Qiagen, Hilden, Germany). A RT2 Profiler PCR Array Format E 384 (4 x 96) was used to be able to screen 4 different samples on one plate. The 384 (4 x 96) option contained 4 replicate primer assay for each 84 pathway-focused

genes and 5 replicate primer assay for each housekeeping (reference) gene; beta-Actin (*ACTB*), beta-2-Microglobulin (*B2M*), Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), Hypoxanthine phosphoribosyltransferase 1 (*HPRT1*), and Ribosomal protein, large, P0 (*RPLP0*). Assays for 5 housekeeping genes enabled normalization of data. Control and each test group were run in a triplicate (n=3, 12 samples total). We used a real-time cycler Quant Studio 5 Applied Biosystems (Foster City, CA, USA). Cycling conditions were: 95.0°C for 10:00 minutes for Stage 1; Stage 2, 95.0°C for 15 seconds followed by 60.0°C for 1:00 minute with 40 repeats (40 cycles) and the Dissociation Stage 3. CT values were exported to an Excel file to create a table of CT values (Supplemental Tables S2 and S5). These tables were then uploaded on to the data analysis web portal at <http://www.qiagen.com/geneglobe>. Samples were assigned to controls (Control) and test groups: PG/VG (Group 1), PG/VG+N (Group 2), PG/VG+N+F (Group 3). The CT cut-off was set to 35. CT values were normalized based on a Manual Selection of reference (housekeeping) genes. The data analysis web portal calculated fold change/regulation using delta delta CT method, in which delta CT was calculated between gene of interest (GOI) and an average of reference genes (HKG), followed by delta-delta CT calculations (delta CT (Test Group)-delta CT (Control Group)). Fold change was then calculated using $2^{(-\text{delta delta CT})}$ formula. Fold-Regulation represents fold-change results in a biologically meaningful way. Fold-change values greater than one indicate a positive- or an up-regulation, and the fold-regulation was equal to the fold-change. Fold-change values less than one indicate a negative or down-regulation, and the fold-regulation was the negative inverse of the fold-change (Supplemental Tables S3 and S6). The fold-change threshold was set to 2. The p-

values were calculated based on a Student's t-test of the replicate $2^{(-\Delta\Delta CT)}$ values for each gene in the control group and treatment groups, and p-values less than 0.05 were considered as significant. The p-value calculation used was based on parametric, unpaired, two-sample equal variance, two-tailed distribution.

Statistical analysis. One-Way ANOVA of Variance for Independent or Correlated Samples analysis along with Tukey HSD test were used to confirm statistical significance in gene expression [$p \leq 0.05$ (*) and $p \leq 0.01$ (**)] in structural and functional epithelial genes. For Human Cytokines and Chemokine expression and Fatty Acid metabolism expression, the p-values were calculated based on a Student's t-test of the replicate $2^{(-\Delta\Delta CT)}$ values for each gene in the control group and treatment groups, and p-values less than 0.05 were considered as significant. The p-value calculation used was based on parametric, unpaired, two-sample equal variance, two-tailed distribution.

Author contributions

V.L. and S.L.T. designed research; V.L. performed experiments; S.L.T supervised the work; V.L and S.L.T wrote the manuscript. Authors approved the final manuscript.

Ethical statement

All stem cell work in this investigation was approved by the Stem Cell Research Oversight Committee at the University of Wisconsin Madison (SC-2015-0008).

802 **Acknowledgement**

803 This work was funded the grants NIH-NIDCD R01 04336 and R01 012773. We
 804 gratefully acknowledge Sierra Raglin for her expert assistance with the construct
 805 sample preparation for this study. We also gratefully acknowledge Stephanie Bartley for
 806 her assistance in ECVE preparation.

807

808

809

References:

1. Ghinai I, Pray IW, Navon L, O’Laughlin K, Saathoff-Huber L, Hoots B, et al. E-cigarette Product Use, or Vaping, Among Persons with Associated Lung Injury - Illinois and Wisconsin, April-September 2019. MMWR Morb Mortal Wkly Rep. 2019;68(39):865–9.
2. Layden JE, Ghinai I, Pray I, Kimball A, Layer M, Tenforde MW, et al. Pulmonary illness related to e-cigarette use in Illinois and Wisconsin - Final report. N Engl J Med. 2020;382(10):903–16.
3. Davidson K, Brancato A, Heetderks P, Mansour W, Matheis E, Nario M, et al. Outbreak of Electronic-Cigarette – Associated Acute Lipoid Pneumonia —. MMWR Morb Mortal Wkly Rep [Internet]. 2019;68(August):784–6. Available from: <https://www.cdc.gov/mmwr/volumes/68/wr/mm6836e1.htm>
4. Dicipinigitis P V., Trachuk P, Fakier F, Teka M, Suhrlund MJ. Vaping-Associated Acute Respiratory Failure Due to Acute Lipoid Pneumonia. Lung [Internet]. 2020;198(1):31–3. Available from: <https://doi.org/10.1007/s00408-019-00277-6>
5. Itoh M, Aoshiba K, Herai Y, Nakamura H, Takemura T. Lung injury associated with electronic cigarettes inhalation diagnosed by transbronchial lung biopsy. Respirol Case Reports. 2018;6(1):1–3.
6. Blount BC, Karwowski MP, Shields PG, Morel-Espinosa M, Valentin-Blasini L, Gardner M, et al. Vitamin E acetate in bronchoalveolar-lavage fluid associated with EVALI. N Engl J Med. 2020;382(8):697–705.
7. Madison MC, Landers CT, Gu BH, Chang CY, Tung HY, You R, et al. Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of

nicotine. *J Clin Invest.* 2019;129(10):4290–304.

8. Chand HS, Muthumalage T, Maziak W, Rahman I. Pulmonary toxicity and the pathophysiology of electronic cigarette, or vaping product, use associated lung injury. *Front Pharmacol.* 2020;10(January):1–7.

9. Mirbolouk M, Charkhchi P, Kianoush S, Uddin SMI, Orimoloye OA, Jaber R, et al. Prevalence and distribution of e-cigarette use among U.S. adults: Behavioral risk factor surveillance system, 2016. *Ann Intern Med.* 2018;169(7):429–38.

10. Sosnowski TR, Odziomek M. Particle size dynamics: Toward a better understanding of electronic cigarette aerosol interactions with the respiratory system. *Front Physiol.* 2018;9(JUL):1–8.

11. Gotts JE, Jordt SE, McConnell R, Tarran R. What are the respiratory effects of e-cigarettes? *BMJ.* 2019;366:1–16.

12. Levendoski EE, Leydon C, Thibeault SL. Vocal Fold Epithelial Barrier in Health and Injury: A Research Review. *J Speech, Lang Hear Res.* 2014;57(3):1679–91.

13. Kivekäs I, Rautiainen M. Epiglottitis, Acute Laryngitis and Croup. In: Durand ML, Deschler DG, editors. *Infections of the Ears, Nose, Throat, and Sinuses.* Springer International Publishing AG, part of Springer Nature 2018; 2018. p. 1–393.

14. Bozzella MJ, Magyar M, DeBiasi RL, Ferrer K. Epiglottitis Associated with Intermittent E-cigarette Use: The vagaries of vaping toxicity. *Pediatrics.* 2020;145(3).

15. Lungova V, Chen X, Wang Z, Christina K, Thibeault SL. Human induced pluripotent stem cell-derived vocal fold mucosa mimics development and responses to smoke exposure. *Nat Commun [Internet].* 2019; Available from:

<http://dx.doi.org/10.1038/s41467-019-12069-w>

16. Griffith JW, Sokol CL, Luster AD. Chemokines and Chemokine Receptors: Positioning Cells for Host Defense and Immunity. *Annu Rev Immunol*. 2014;32(1):659–702.
17. Lim KS, Yong ZWE, Wang H, Tan TZ, Huang RYJ, Yamamoto D, et al. Inflammatory and mitogenic signals drive interleukin 23 subunit alpha (IL23A) secretion independent of IL12B in intestinal epithelial cells. *J Biol Chem*. 2020;295(19):6387–400.
18. Russell MA, Morgan NG. The impact of anti-inflammatory cytokines on the pancreatic β -cell. *Islets*. 2014;6(3):1–10.
19. Glaser L, Coulter PJ, Shields M, Touzelet O, Power UF, Broadbent L. Airway epithelial derived cytokines and chemokines and their role in the immune response to respiratory syncytial virus infection. *Pathogens*. 2019;8(3).
20. Jensen KK, Previs SF, Zhu L, Herath K, Wang SP, Bhat G, et al. Demonstration of diet-induced decoupling of fatty acid and cholesterol synthesis by combining gene expression array and 2H 2O quantification. *Am J Physiol - Endocrinol Metab*. 2012;302(2).
21. Grevengoed TJ, Klett EL, Coleman RA. Acyl-CoA Metabolism and Partitioning. *Annu Rev Nutr* [Internet]. 2014;34:1–30. Available from: doi:10.1146/annurev-nutr-071813-105541
22. Schönfeld P, Wojtczak L. Short- and medium-chain fatty acids in energy metabolism: The cellular perspective. *J Lipid Res*. 2016;57(6):943–54.
23. Schwenk RW, Holloway GP, Luiken JJFP, Bonen A, Glatz JFC. Fatty acid

transport across the cell membrane: Regulation by fatty acid transporters.

Prostaglandins Leukot Essent Fat Acids [Internet]. 2010;82(4–6):149–54.

Available from: <http://dx.doi.org/10.1016/j.plefa.2010.02.029>

24. Salturk Z, Çakir Ç, Sünnetçi G, Atar Y, Kumral TL, Yildirim G, et al. Effects of Electronic Nicotine Delivery System on Larynx: Experimental Study. *J Voice*. 2015;29(5):560–3.

25. Reidel B, Radicioni G, Clapp PW, Ford AA, Abdelwahab S, Rebuli ME, et al. E-cigarette use causes a unique innate immune response in the lung, involving increased neutrophilic activation and altered mucin secretion. *Am J Respir Crit Care Med*. 2018;197(4):492–501.

26. Martin EM, Clapp PW, Rebuli ME, Pawlak EA, Glista-Baker E, Benowitz NL, et al. E-cigarette use results in suppression of immune and inflammatory-response genes in nasal epithelial cells similar to cigarette smoke. *Am J Physiol - Lung Cell Mol Physiol*. 2016;311(1):L135–44.

27. Sommerfeld CG, Weiner DJ, Nowalk A, Larkin A. Hypersensitivity pneumonitis and acute respiratory distress syndrome from e-cigarette use. *Pediatrics*. 2018;141(6).

28. Leydon C, Bartlett RS, Roenneburg DA, Thibeault SL. Localization of Label-Retaining Cells. *J Speech, Lang Hear Res*. 2011;54:1060–6.

29. Samuels TL, Handler E, Syring ML, Pajewski NM, Blumin JH, Kerschner JE, et al. Mucin gene expression in human laryngeal epithelia: Effect of laryngopharyngeal reflux. *Ann Otol Rhinol Laryngol*. 2008;117(9):688–95.

30. Leonardi R, Perrotta RE, Loreto C, Musumeci G, Crimi S, Dos Santos JN, et al.

- 902 Toll-like receptor 4 expression in the epithelium of inflammatory periapical lesions.
903 An immunohistochemical study. *Eur J Histochem*. 2015;59(4):1–4.
- 904 31. Günther J, Seyfert HM. The first line of defence: insights into mechanisms and
905 relevance of phagocytosis in epithelial cells. *Semin Immunopathol*.
906 2018;(September).
- 907 32. Higham A, Bostock D, Booth G, Dungwa J V., Singh D. The effect of electronic
908 cigarette and tobacco smoke exposure on COPD bronchial epithelial cell
909 inflammatory responses. *Int J COPD*. 2018;13:989–1000.
- 910 33. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: Role in metabolic
911 diseases and potential as drug targets. *Nat Rev Drug Discov*. 2008;7(6):489–503.
- 912 34. Dharmarajan S, Newberry EP, Montenegro G, Nalbantoglu Ilk, Davis VR,
913 Clanahan MJ, et al. Liver fatty acid-binding protein (L-Fabp) modifies intestinal
914 fatty acid composition and adenoma formation in ApcMin/+ mice. *Cancer Prev*
915 *Res*. 2013;6(10):1026–37.
- 916 35. Martin GG, Atshaves BP, McIntosh AL, Mackie JT, Kier AB, Schroeder F. Liver
917 fatty acid binding protein gene ablation potentiates hepatic cholesterol
918 accumulation in cholesterol-fed female mice. *Am J Physiol - Gastrointest Liver*
919 *Physiol*. 2006;290(1):36–48.
- 920 36. Titze IR. Physiologic And Acoustic Differences Between Male And Female
921 Voices. *J Acoust Soc Am*. 1989;85(4):1699–707.
- 922 37. Garnier M, Henrich N, Smith J, Wolfe J. Vocal tract adjustments in the high
923 soprano range. *J Acoust Soc Am*. 2010;127(6):3771–80.
- 924 38. Cohen SM, Kim J, Roy N, Asche C, Courey M. The impact of laryngeal disorders

- 925 on work-related dysfunction. *Laryngoscope*. 2012;122(7):1589–94.
- 926 39. Cohen SM, Kim J, Roy N, Asche C, Courey M. Direct health care costs of
- 927 laryngeal diseases and disorders. *Laryngoscope*. 2012;122(7):1582–8.
- 928 40. Roy N, Merrill RM, Gray SD, Smith EM. Voice disorders in the general population:
- 929 Prevalence, risk factors, and occupational impact. *Laryngoscope*.
- 930 2005;115(11):1988–95.
- 931 41. Leydon C, Selekman JA, Palecek S, Thibeault SL. Human Embryonic Stem Cell-
- 932 Derived Epithelial Cells in a Novel In Vitro Model of Vocal Mucosa. *TISSUE Eng*
- 933 Part A Vol 19, Numbers 19 20, 2013. 2013;19.
- 934

Supplemental Table S1: Table of genes analyzed in the RT² Profiler PCR Arrays --Human Cytokine and Chemokines (PAHS-150Z)

Position	RefSeq Number	Symbol	Description
A01	NM_004797	ADIPOQ	Adiponectin, C1Q and collagen domain containing
A02	NM_001200	BMP2	Bone morphogenetic protein 2
A03	NM_130851	BMP4	Bone morphogenetic protein 4
A04	NM_001718	BMP6	Bone morphogenetic protein 6
A05	NM_001719	BMP7	Bone morphogenetic protein 7
A06	NM_001735	C5	Complement component 5
A07	NM_002981	CCL1	Chemokine (C-C motif) ligand 1
A08	NM_002986	CCL11	Chemokine (C-C motif) ligand 11
A09	NM_005408	CCL13	Chemokine (C-C motif) ligand 13
A10	NM_002987	CCL17	Chemokine (C-C motif) ligand 17
A11	NM_002988	CCL18	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
A12	NM_006274	CCL19	Chemokine (C-C motif) ligand 19
B01	NM_002982	CCL2	Chemokine (C-C motif) ligand 2
B02	NM_004591	CCL20	Chemokine (C-C motif) ligand 20
B03	NM_002989	CCL21	Chemokine (C-C motif) ligand 21
B04	NM_002990	CCL22	Chemokine (C-C motif) ligand 22
B05	NM_002991	CCL24	Chemokine (C-C motif) ligand 24
B06	NM_002983	CCL3	Chemokine (C-C motif) ligand 3
B07	NM_002985	CCL5	Chemokine (C-C motif) ligand 5
B08	NM_006273	CCL7	Chemokine (C-C motif) ligand 7
B09	NM_005623	CCL8	Chemokine (C-C motif) ligand 8

B10	NM_000074	CD40LG	CD40 ligand
B11	NM_000614	CNTF	Ciliary neurotrophic factor
B12	NM_000757	CSF1	Colony stimulating factor 1 (macrophage)
C01	NM_000758	CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
C02	NM_000759	CSF3	Colony stimulating factor 3 (granulocyte)
C03	NM_002996	CX3CL1	Chemokine (C-X3-C motif) ligand 1
C04	NM_001511	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
C05	NM_001565	CXCL10	Chemokine (C-X-C motif) ligand 10
C06	NM_005409	CXCL11	Chemokine (C-X-C motif) ligand 11
C07	NM_000609	CXCL12	Chemokine (C-X-C motif) ligand 12
C08	NM_006419	CXCL13	Chemokine (C-X-C motif) ligand 13
C09	NM_022059	CXCL16	Chemokine (C-X-C motif) ligand 16
C10	NM_002089	CXCL2	Chemokine (C-X-C motif) ligand 2
C11	NM_002994	CXCL5	Chemokine (C-X-C motif) ligand 5
C12	NM_002416	CXCL9	Chemokine (C-X-C motif) ligand 9
D01	NM_000639	FASLG	Fas ligand (TNF superfamily, member 6)
D02	NM_000175	GPI	Glucose-6-phosphate isomerase
D03	NM_000605	IFNA2	Interferon, alpha 2
D04	NM_000619	IFNG	Interferon, gamma
D05	NM_000572	IL10	Interleukin 10
D06	NM_000641	IL11	Interleukin 11
D07	NM_000882	IL12A	Interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)
D08	NM_002187	IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)
D09	NM_002188	IL13	Interleukin 13

D10	NM_000585	IL15	Interleukin 15
D11	NM_004513	IL16	Interleukin 16
D12	NM_002190	IL17A	Interleukin 17A
E01	NM_052872	IL17F	Interleukin 17F
E02	NM_001562	IL18	Interleukin 18 (interferon-gamma-inducing factor)
E03	NM_000575	IL1A	Interleukin 1, alpha
E04	NM_000576	IL1B	Interleukin 1, beta
E05	NM_000577	IL1RN	Interleukin 1 receptor antagonist
E06	NM_000586	IL2	Interleukin 2
E07	NM_021803	IL21	Interleukin 21
E08	NM_020525	IL22	Interleukin 22
E09	NM_016584	IL23A	Interleukin 23, alpha subunit p19
E10	NM_006850	IL24	Interleukin 24
E11	NM_145659	IL27	Interleukin 27
E12	NM_000588	IL3	Interleukin 3 (colony-stimulating factor, multiple)
F01	NM_000589	IL4	Interleukin 4
F02	NM_000879	IL5	Interleukin 5 (colony-stimulating factor, eosinophil)
F03	NM_000600	IL6	Interleukin 6 (interferon, beta 2)
F04	NM_000880	IL7	Interleukin 7
F05	NM_000584	CXCL8	Interleukin 8
F06	NM_000590	IL9	Interleukin 9
F07	NM_002309	LIF	Leukemia inhibitory factor (cholinergic differentiation factor)
F08	NM_000595	LTA	Lymphotoxin alpha (TNF superfamily, member 1)
F09	NM_002341	LTB	Lymphotoxin beta (TNF superfamily, member 3)
F10	NM_002415	MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)
F11	NM_005259	MSTN	Myostatin

F12	NM_018055	NODAL	Nodal homolog (mouse)
G01	NM_020530	OSM	Oncostatin M
G02	NM_002704	PPBP	Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)
G03	NM_000582	SPP1	Secreted phosphoprotein 1
G04	NM_003238	TGFB2	Transforming growth factor, beta 2
G05	NM_000460	THPO	Thrombopoietin
G06	NM_000594	TNF	Tumor necrosis factor
G07	NM_002546	TNFRSF11B	Tumor necrosis factor receptor superfamily, member 11b
G08	NM_003810	TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10
G09	NM_003701	TNFSF11	Tumor necrosis factor (ligand) superfamily, member 11
G10	NM_006573	TNFSF13B	Tumor necrosis factor (ligand) superfamily, member 13b
G11	NM_003376	VEGFA	Vascular endothelial growth factor A
G12	NM_002995	XCL1	Chemokine (C motif) ligand 1
H01	NM_001101	ACTB	Actin, beta
H02	NM_004048	B2M	Beta-2-microglobulin
H03	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H04	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1
H05	NM_001002	RPLP0	Ribosomal protein, large, P0
H06	SA_00105	HGDC	Human Genomic DNA Contamination
H07	SA_00104	RTC	Reverse Transcription Control
H08	SA_00104	RTC	Reverse Transcription Control
H09	SA_00104	RTC	Reverse Transcription Control
H10	SA_00103	PPC	Positive PCR Control
H11	SA_00103	PPC	Positive PCR Control
H12	SA_00103	PPC	Positive PCR Control

Supplemental Table S2: List of CT values of human cytokines and chemokines uploaded on to the data analysis web portal at <http://www.qiagen.com/geneglobe>

				PG/VG	PG/VG	PG/VG	PG/VG+N	PG/VG+N	PG/VG+N	PG/VG+N	PG/VG+N	PG/VG+N
	Control	Control	Contr	Test	Test	Test	Test	Test	Test	Test	Test	Test
	Group	Group	ol	Group	Group	Group	Group	Group	Group	Group	Group	Group
			ol	1	1	1	2	2	2	3	3	3
			Group									
			p									
A01	34.69	35.26	33.19	32.75	35.37	33.31	34.02	34.20	33.79	34.18	34.83	34.92
A02	26.76	30.27	27.15	29.55	29.60	26.59	27.40	28.55	27.19	27.15	29.63	26.95
A03	27.22	28.81	27.23	25.69	26.43	23.47	25.81	28.06	27.14	26.52	28.30	26.62
A04	28.82	29.82	28.60	29.21	28.57	26.89	26.97	28.59	28.68	27.31	28.92	27.74
A05	28.90	28.23	26.85	26.13	26.45	24.38	26.76	28.28	27.24	28.05	30.05	28.34
A06	28.75	40	28.14	30.70	40	27.85	27.24	28.81	29.23	26.98	28.71	27.65
A07	30.55	40	32.39	30.43	30.58	29.29	29.08	30.20	29.47	28.94	29.81	29.82
A08	32.12	33.44	31.04	34.58	31.63	30.66	27.92	29.42	29.51	28.08	30.06	28.46
A09	31.70	31.66	30.35	30.38	30.40	29.59	28.64	30.60	29.81	29.84	29.61	29.07
A10	33.58	33.11	33.38	32.00	34.11	31.91	33.22	34.29	32.55	34.68	33.79	32.24
A11	32.61	30.82	32.93	31.83	30.22	31.36	32.89	29.98	31.27	33.59	30.44	33.80
A12	37.71	35.72	31.67	34.27	32.71	31.91	33.47	31.44	35.16	36.19	31.72	33.57
B01	26.31	27.03	25.41	24.86	24.83	23.13	23.62	25.97	24.01	24.33	26.58	24.55
B02	27.14	27.90	27.27	27.90	27.18	25.89	26.11	27.45	26.32	25.53	26.29	25.60
B03	31.60	33.37	32.96	34.65	33.59	32.41	31.46	31.89	32.63	32.38	32.84	31.81
B04	40	34.60	40	40	36.92	40	35.05	40	40	40	40	33.58
B05	34.80	33.22	34.10	33.26	30.81	32.57	32.66	35.59	32.79	33.09	30.72	32.94
B06	33.99	34.21	33.55	34.55	33.68	33.14	32.85	33.28	32.68	33.28	33.19	32.84
B07	32.49	32.86	31.82	32.85	32.29	31.36	30.37	31.93	31.26	32.18	33.77	32.77
B08	29.00	29.61	28.18	29.80	30.20	29.58	26.61	28.27	27.60	29.39	27.14	27.17
B09	35.71	35.74	35.50	34.23	34.48	32.28	34.46	33.74	33.73	33.68	33.67	35.51
B10	32.59	31.98	32.54	31.75	32.06	31.27	31.28	32.28	31.50	31.46	32.41	32.49
B11	29.36	29.38	29.13	29.48	28.88	27.94	28.87	29.16	29.66	30.49	29.48	29.47
B12	27.58	26.34	25.37	26.64	26.29	25.26	25.02	24.98	25.14	25.17	25.30	23.84

C01	30.04	29.61	30.59	27.91	29.20	30.54	28.20	29.29	29.47	28.85	29.24	30.28
C02	31.53	32.41	31.33	31.79	32.19	29.95	30.91	32.20	31.73	31.95	31.89	30.53
C03	28.92	29.56	28.97	28.84	30.63	28.12	27.62	29.09	28.18	28.60	29.43	28.72
C04	26.25	29.59	27.53	28.44	29.79	24.64	22.66	27.25	25.51	23.06	27.57	23.45
C05	32.76	32.11	30.85	33.47	29.10	30.00	29.92	30.96	30.65	29.99	30.05	29.40
C06	36.42	37.32	36.16	34.73	35.41	33.67	36.53	36.50	35.35	36.69	36.15	34.88
C07	23.23	23.75	23.00	24.51	24.79	23.41	23.40	22.57	22.85	21.34	22.92	21.99
C08	29.72	29.75	28.96	30.60	29.80	29.50	27.66	28.12	27.86	26.47	27.91	27.49
C09	37.66	36.07	34.16	35.91	33.78	33.03	33.14	34.25	33.88	35.90	33.66	33.99
C10	27.44	28.00	24.67	25.93	26.00	23.51	24.99	24.43	24.28	27.04	24.57	22.52
C11	33.62	34.44	34.09	34.08	32.30	32.65	32.65	32.99	32.92	33.74	33.19	31.47
C12	31.79	32.27	31.26	31.50	30.75	30.50	30.75	31.32	30.51	29.62	31.00	30.59
D01	31.68	31.65	32.16	31.35	30.74	31.38	30.01	30.38	30.87	30.00	30.91	33.19
D02	23.28	23.57	23.22	22.99	22.27	20.86	21.10	22.92	21.31	20.93	22.88	21.18
D03	35.13	36.09	35.42	36.60	36.51	33.96	34.41	36.34	36.32	35.51	36.41	35.33
D04	32.53	33.45	34.08	35.35	32.99	32.45	34.43	34.22	33.17	34.60	34.72	34.32
D05	32.18	30.45	29.97	34.77	26.93	30.70	31.59	32.13	32.59	31.38	26.25	31.50
D06	27.90	27.21	26.23	29.10	29.20	27.85	25.57	27.13	27.48	25.22	26.82	25.84
D07	32.28	32.23	30.51	31.56	30.61	29.09	29.99	32.27	31.39	32.72	31.94	30.47
D08	32.98	32.71	33.84	33.35	33.23	31.62	32.70	33.10	32.51	32.17	33.41	31.25
D09	29.46	30.85	30.76	30.34	29.78	29.09	30.62	30.54	29.22	29.01	29.85	30.78
D10	28.45	28.34	27.72	28.88	28.28	27.96	27.22	26.89	27.50	27.74	26.87	26.42
D11	33.14	31.93	31.33	33.58	33.68	31.74	32.69	31.84	31.25	33.41	33.81	29.95
D12	36.91	35.22	35.38	36.81	34.49	34.49	34.62	38.03	36.07	37.01	33.77	34.46
E01	30.27	30.25	30.95	30.19	30.12	29.52	29.60	29.41	29.69	30.14	30.27	30.19
E02	29.96	31.01	31.34	32.18	31.24	29.98	30.14	31.57	29.35	29.50	30.67	30.09
E03	31.12	32.52	32.37	33.02	31.78	29.82	29.96	31.31	31.20	31.11	31.97	30.64
E04	27.69	30.92	29.21	32.43	32.85	30.84	27.44	30.11	30.28	28.57	30.95	29.31
E05	29.86	28.82	28.16	29.63	30.24	27.56	25.96	28.20	27.74	26.42	28.38	26.85
E06	35.88	35.56	35.73	34.64	33.93	33.78	34.00	33.90	34.06	33.84	34.23	34.88
E07	24.00	25.26	25.19	26.98	26.51	26.55	26.81	25.98	25.29	26.63	26.51	26.44
E08	28.36	28.79	28.92	28.78	29.60	28.96	25.99	29.69	28.61	29.86	29.95	28.73
E09	29.40	30.66	29.45	33.18	32.12	30.32	28.69	31.15	30.55	31.48	31.68	30.61
E10	30.00	29.53	28.33	29.57	29.35	27.64	27.99	28.23	27.91	28.82	27.55	25.94

E11	31.80	31.83	31.39	32.06	31.32	29.78	30.74	31.02	30.13	31.27	31.38	30.59
E12	34.25	29.91	32.65	33.99	28.34	30.18	32.31	27.56	32.11	33.62	25.98	31.14
F01	29.45	30.53	30.19	29.00	31.35	30.66	29.92	30.81	30.07	29.87	30.83	30.27
F02	32.76	34.80	34.81	32.64	34.19	33.44	32.34	33.41	33.10	32.41	34.69	34.51
F03	23.57	23.94	23.02	23.71	23.80	22.13	19.99	21.60	21.41	19.59	21.01	19.87
F04	32.75	34.61	35.17	33.74	34.10	34.61	33.92	36.58	33.90	34.04	36.51	33.50
F05	24.58	24.33	22.83	23.90	24.71	22.33	20.48	23.06	22.69	20.56	23.09	20.86
F06	35.65	36.91	35.37	37.16	36.05	34.95	34.30	38.13	36.37	34.37	37.45	35.98
F07	29.33	28.44	26.67	29.57	28.78	26.71	24.88	27.65	27.37	28.76	26.97	26.00
F08	32.08	33.27	32.46	34.49	31.77	31.00	32.72	32.45	31.69	31.99	32.42	31.32
F09	32.38	32.49	31.81	32.09	32.39	30.21	31.85	32.58	31.14	32.33	32.77	31.46
F10	22.40	21.56	20.25	20.82	21.02	19.83	21.19	21.57	20.50	20.57	19.90	19.57
F11	32.24	32.78	31.74	31.92	32.14	29.95	31.90	32.32	31.05	31.55	32.29	31.74
F12	33.28	33.83	31.82	33.39	32.83	30.64	31.70	32.14	31.68	34.11	33.56	30.82
G01	32.62	32.32	33.60	33.20	33.76	31.20	31.31	30.99	31.82	31.84	31.19	33.61
G02	30.70	32.92	31.90	34.04	33.29	32.93	32.93	31.32	34.35	30.91	29.23	30.97
G03	25.93	26.61	25.82	27.17	27.15	25.97	24.33	25.92	25.89	27.63	27.35	26.62
G04	26.16	26.86	26.10	25.75	25.46	23.84	23.96	25.50	26.00	24.15	26.02	24.90
G05	30.20	31.47	30.63	31.25	30.85	29.55	29.82	30.83	30.61	29.37	31.17	29.14
G06	40	35.58	34.07	10.58	34.42	40	35.52	40	38.63	33.45	32.87	33.30
G07	28.38	29.40	26.67	28.71	31.66	25.89	22.30	27.14	26.61	25.96	26.76	24.13
G08	31.23	30.44	30.00	29.30	29.52	28.40	28.29	30.82	29.78	29.46	30.85	30.11
G09	32.88	31.61	31.41	31.34	31.28	29.63	30.51	31.95	31.44	31.72	32.50	31.33
G10	28.63	27.84	27.61	29.38	28.89	27.88	27.64	27.17	27.62	28.26	27.31	26.59
G11	24.36	24.46	23.44	25.53	23.13	22.22	24.36	24.19	23.83	24.19	23.21	22.33
G12	35.76	35.57	35.57	37.72	31.79	31.78	37.69	32.51	36.57	38.26	31.15	32.88
H01	20.61	23.63	20.89	21.00	22.56	17.22	18.59	22.03	19.25	19.56	22.22	19.52
H02	20.60	22.05	20.97	21.73	21.13	20.09	17.46	20.20	18.46	18.33	20.24	18.79
H03	20.37	22.68	20.18	21.63	19.69	19.69	19.13	19.86	21.15	19.50	19.79	19.50
H04	26.30	26.68	25.93	26.23	25.80	25.16	24.96	26.13	26.35	25.25	26.14	25.32
H05	22.68	21.40	20.23	19.92	19.64	18.31	16.57	19.10	20.49	17.44	19.49	17.63
H06	35.22	35.67	36.41	34.31	34.93	34.44	35.26	35.75	35.50	35.17	35.68	34.62
H07	22.40	23.64	23.88	23.71	25.29	23.19	21.12	25.39	26.30	21.13	24.90	22.89
H08	22.50	23.83	24.00	24.51	25.68	22.81	21.40	25.11	25.96	21.22	24.80	22.732

H09	22.59	23.77	23.65	24.38	25.36	22.84	25.84	24.87	23.68	26.77	25.12	22.97
H10	27.66	23.23	27.91	27.13	23.89	25.71	28.59	23.52	26.43	28.30	22.87	24.21
H11	28.17	23.90	27.31	28.16	22.66	23.92	29.17	24.15	25.87	27.64	22.27	23.71
H12	28.80	22.92	27.84	27.73	23.81	24.25	29.33	23.82	26.68	28.15	22.17	23.90

Supplemental Table S3: Fold regulation and p-values of genes associated mucosal inflammation. Fold regulation is compared to controls

Position	Gene Symbol	Group 1		Group 2		Group 3	
		Fold Regulation	p-Value	Fold Regulation	p-Value	Fold Regulation	p-Value
A01	ADIPOQ	-1.34	0.949612	-2.65	0.07756	-4.34	0.022261
A02	BMP2	-2.93	0.162542	-2.56	0.214097	-3.07	0.150726
A03	BMP4	2.89	0.048146	-1.93	0.019491	-2.22	0.007207
A04	BMP6	-1.13	0.333216	-1.62	0.024908	-1.6	0.015384
A05	BMP7	2.49	0.044874	-2.19	0.15756	-5.99	0.051444
A06	C5	-2.99	0.26999	1.42	0.521938	2.12	0.946686
A07	CCL1	2.87	0.296137	2.57	0.391479	2.56	0.40323
A08	CCL11	-2.17	0.211494	2.93	0.009991	2.97	0.003055
A09	CCL13	1.06	0.855139	-1.1	0.634625	-1	0.89173
A10	CCL17	-1.27	0.847791	-3.24	0.228373	-3.95	0.188997
A11	CCL18	-1.03	0.717673	-1.94	0.633077	-4.78	0.471747
A12	CCL19	-1.08	0.653829	-2.16	0.832994	-2.47	0.749384
B01	CCL2	1.93	0.005131	1.01	0.765233	-1.59	0.049993
B02	CCL20	-1.49	0.161355	-1.85	0.180155	-1.1	0.733896
B03	CCL21	-3.81	0.076845	-2.06	0.224412	-2.76	0.11197
B04	CCL22	-2.23	0.29771	-3.56	0.192872	-2.69	0.229169
B05	CCL24	1.74	0.560675	-2.2	0.38765	1.02	0.733473
B06	CCL3	-1.87	0.158638	-1.65	0.368825	-1.93	0.216966
B07	CCL5	-1.75	0.090679	-1.41	0.254146	-4.87	0.010946
B08	CCL7	-3.88	0.008	-1.2	0.425252	-1.66	0.946204
B09	CCL8	1.24	0.778056	-1.6	0.610014	-1.85	0.510117
B10	CD40LG	-1.28	0.57854	-2.02	0.329164	-2.86	0.238628
B11	CNTF	-1.41	0.364815	-3.11	0.121358	-4.88	0.090452
B12	CSF1	-1.57	0.295875	-1.24	0.661535	-1.08	0.72296
C01	CSF2	-1.12	0.769752	-1.52	0.413178	-2.21	0.358839
C02	CSF3	-1.5	0.0961	-2.94	0.007822	-2.76	0.021024
C03	CX3CL1	-2.11	0.219691	-1.8	0.153513	-2.89	0.038237
C04	CXCL1	-1.82	0.646625	1.93	0.39189	2.51	0.224539

C05	CXCL10	1.02	0.615737	-1.23	0.522227	1.25	0.685628
C06	CXCL11	-1.41	0.413821	-3.25	0.14523	-3.31	0.134927
C07	CXCL12	-3.83	0.02162	-2.49	0.18512	-1.44	0.200292
C08	CXCL13	-2.86	0.074479	-1.07	0.980486	1.34	0.426084
C09	CXCL16	-1.18	0.626293	-1.67	0.221699	-2.4	0.319296
C10	CXCL2	1.44	0.761869	1.35	0.610594	1.17	0.492224
C11	CXCL5	1.01	0.81883	-1.42	0.487189	-1.43	0.658705
C12	CXCL9	-1.13	0.838286	-1.72	0.288454	-1.31	0.369544
D01	FASLG	-1.28	0.71466	-1.22	0.660594	-2.46	0.386366
D02	GPI	1.22	0.645905	-1.08	0.946249	-1.05	0.721945
D03	IFNA2	-1.6	0.316888	-2.84	0.149693	-3.4	0.133243
D04	IFNG	-2.22	0.203707	-4.87	0.106166	-7.76	0.068464
D05	IL10	-1.95	0.562303	-7.67	0.137885	-1.53	0.501363
D06	IL11	-6.18	0.062115	-2.48	0.13037	-1.53	0.280914
D07	IL12A	1.17	0.723315	-2.36	0.071131	-3.48	0.071281
D08	IL12B	-1.5	0.380721	-2.45	0.284113	-1.82	0.332568
D09	IL13	-1.33	0.399013	-2.76	0.212357	-2.44	0.181454
D10	IL15	-2.34	0.136928	-1.66	0.493474	-1.52	0.603639
D11	IL16	-3.72	0.139453	-2.81	0.272895	-4.06	0.244233
D12	IL17A	-1.61	0.392563	-2.97	0.147803	-2.26	0.414697
E01	IL17F	-1.4	0.462483	-1.71	0.43161	-2.77	0.209698
E02	IL18	-2.62	0.111179	-2.43	0.273233	-2.11	0.153393
E03	IL1A	-1.47	0.328236	-1.43	0.303056	-2	0.144028
E04	IL1B	-13.84	0.135316	-3.26	0.214877	-4.29	0.184107
E05	IL1RN	-2.34	0.19862	-1.04	0.648971	-1.03	0.673274
E06	IL2	-1.11	0.78615	-1.61	0.473853	-2.12	0.285518
E07	IL21	-7.39	0.041969	-7.5	0.050909	-11.12	0.035929
E08	IL22	-2.73	0.169701	-2.15	0.199245	-6.02	0.062789
E09	IL23A	-8.35	0.001218	-3.98	0.002164	-9.1	0.001272
E10	IL24	-1.51	0.238463	-1.37	0.578921	1.06	0.614469
E11	IL27	-1.33	0.380075	-1.57	0.533096	-2.25	0.15919
E12	IL3	1.33	0.95802	-1.06	0.815109	1.2	0.484133
F01	IL4	-2.48	0.566347	-3.77	0.046445	-4.1	0.035901

F02	IL5	-1.26	0.866017	-1.44	0.388842	-2.85	0.189438
F03	IL6	-1.66	0.108928	1.75	0.049729	3.01	0.001628
F04	IL7	-2.08	0.337616	-3.61	0.146737	-3.55	0.134236
F05	CXCL8	-1.69	0.20717	1.1	0.942692	1.57	0.262679
F06	IL9	-2.01	0.281961	-2.77	0.1509	-2.94	0.14391
F07	LIF	-2.35	0.144571	-1.14	0.521211	-1.82	0.501239
F08	LTA	-1.79	0.352771	-2.61	0.128913	-2.1	0.06198
F09	LTB	-1.28	0.403547	-2.51	0.184707	-3.31	0.055584
F10	MIF	-1.13	0.592163	-2.6	0.184788	-1.3	0.683908
F11	MSTN	-1.08	0.686789	-2.3	0.180819	-2.59	0.024663
F12	NODAL	-1.26	0.392322	-1.47	0.451274	-3.06	0.1661
G01	OSM	-1.87	0.323128	-1.17	0.740635	-2.19	0.552686
G02	PPBP	-6.1	0.0713	-6.65	0.144067	-1.23	0.78631
G03	SPP1	-3.18	0.019935	-1.94	0.054915	-7.17	0.009913
G04	TGFB2	1.26	0.291319	-1.39	0.263056	-1.33	0.261376
G05	THPO	-1.75	0.070771	-2.54	0.037897	-1.85	0.06717
G06	TNF	127.49	0.373901	-4.02	0.050591	-1.22	0.969815
G07	TNFRSF11B	-3.09	0.268953	2.14	0.359668	1.7	0.344565
G08	TNFSF10	1.37	0.659453	-1.71	0.292383	-2.54	0.176298
G09	TNFSF11	1.14	0.847909	-2.05	0.280084	-3.14	0.196984
G10	TNFSF13B	-3.29	0.158789	-2.22	0.331585	-2.18	0.332172
G11	VEGFA	-1.48	0.522086	-3.33	0.065147	-1.89	0.334883
G12	XCL1	2.17	0.328343	-1.83	0.947324	1.17	0.480846
H01	ACTB	1.34	0.498357	1.04	0.823407	-1.4	0.424168
H02	B2M	-1.74	0.069622	1.75	0.198236	1.25	0.304749
H03	GAPDH	-1.22	0.842034	-1.59	0.393738	-1.22	0.668975
H04	HPRT1	-1.37	0.36067	-2.31	0.067216	-2.04	0.08229
H05	RPLP0	2.17	0.136325	2.02	0.292426	2.79	0.046283

Supplemental Table S4: Table of genes involved in the Fatty Acid Metabolism (PAHS-007Z)

Position	RefSeq Number	Symbol	Description
A01	NM_001607	ACAA1	Acetyl-CoA acyltransferase 1
A02	NM_006111	ACAA2	Acetyl-CoA acyltransferase 2
A03	NM_025247	ACAD10	Acyl-CoA dehydrogenase family, member 10
A04	NM_032169	ACAD11	Acyl-CoA dehydrogenase family, member 11
A05	NM_014049	ACAD9	Acyl-CoA dehydrogenase family, member 9
A06	NM_001608	ACADL	Acyl-CoA dehydrogenase, long chain
A07	NM_000016	ACADM	Acyl-CoA dehydrogenase, C-4 to C-12 straight chain
A08	NM_000017	ACADS	Acyl-CoA dehydrogenase, C-2 to C-3 short chain
A09	NM_001609	ACADSB	Acyl-CoA dehydrogenase, short/branched chain
A10	NM_000018	ACADVL	Acyl-CoA dehydrogenase, very long chain
A11	NM_000019	ACAT1	Acetyl-CoA acetyltransferase 1
A12	NM_005891	ACAT2	Acetyl-CoA acetyltransferase 2
B01	NM_001037161	ACOT1	Acyl-CoA thioesterase 1
B02	NM_130767	ACOT12	Acyl-CoA thioesterase 12
B03	NM_001037162	ACOT6	Acyl-CoA thioesterase 6
B04	NM_181866	ACOT7	Acyl-CoA thioesterase 7
B05	NM_005469	ACOT8	Acyl-CoA thioesterase 8
B06	NM_001033583	ACOT9	Acyl-CoA thioesterase 9
B07	NM_004035	ACOX1	Acyl-CoA oxidase 1, palmitoyl
B08	NM_003500	ACOX2	Acyl-CoA oxidase 2, branched chain
B09	NM_003501	ACOX3	Acyl-CoA oxidase 3, pristanoyl
B10	NM_015162	ACSBG1	Acyl-CoA synthetase bubblegum family member 1
B11	NM_030924	ACSBG2	Acyl-CoA synthetase bubblegum family member 2
B12	NM_001995	ACSL1	Acyl-CoA synthetase long-chain family member 1
C01	NM_004457	ACSL3	Acyl-CoA synthetase long-chain family member 3
C02	NM_004458	ACSL4	Acyl-CoA synthetase long-chain family member 4

C03	NM_016234	ACSL5	Acyl-CoA synthetase long-chain family member 5
C04	NM_001009185	ACSL6	Acyl-CoA synthetase long-chain family member 6
C05	NM_005622	ACSM3	Acyl-CoA synthetase medium-chain family member 3
C06	NM_001080454	ACSM4	Acyl-CoA synthetase medium-chain family member 4
C07	NM_017888	ACSM5	Acyl-CoA synthetase medium-chain family member 5
C08	NM_000690	ALDH2	Aldehyde dehydrogenase 2 family (mitochondrial)
C09	NM_004051	BDH1	3-hydroxybutyrate dehydrogenase, type 1
C10	NM_020139	BDH2	3-hydroxybutyrate dehydrogenase, type 2
C11	NM_001876	CPT1A	Carnitine palmitoyltransferase 1A (liver)
C12	NM_004377	CPT1B	Carnitine palmitoyltransferase 1B (muscle)
D01	NM_152359	CPT1C	Carnitine palmitoyltransferase 1C
D02	NM_000098	CPT2	Carnitine palmitoyltransferase 2
D03	NM_000755	CRAT	Carnitine O-acetyltransferase
D04	NM_021151	CROT	Carnitine O-octanoyltransferase
D05	NM_001359	DECR1	2,4-dienoyl CoA reductase 1, mitochondrial
D06	NM_020664	DECR2	2,4-dienoyl CoA reductase 2, peroxisomal
D07	NM_004092	ECHS1	Enoyl CoA hydratase, short chain, 1, mitochondrial
D08	NM_006117	ECI2	Enoyl-CoA delta isomerase 2
D09	NM_001966	EHHADH	Enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase
D10	NM_001443	FABP1	Fatty acid binding protein 1, liver
D11	NM_000134	FABP2	Fatty acid binding protein 2, intestinal
D12	NM_004102	FABP3	Fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)
E01	NM_001442	FABP4	Fatty acid binding protein 4, adipocyte
E02	NM_001444	FABP5	Fatty acid binding protein 5 (psoriasis-associated)
E03	NM_001445	FABP6	Fatty acid binding protein 6, ileal
E04	NM_001446	FABP7	Fatty acid binding protein 7, brain
E05	NM_004104	FASN	Fatty acid synthase
E06	NM_000159	GCDH	Glutaryl-CoA dehydrogenase

E07	NM_000167	GK	Glycerol kinase
E08	NM_033214	GK2	Glycerol kinase 2
E09	NM_005276	GPD1	Glycerol-3-phosphate dehydrogenase 1 (soluble)
E10	NM_000408	GPD2	Glycerol-3-phosphate dehydrogenase 2 (mitochondrial)
E11	NM_000182	HADHA	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit
E12	NM_000191	HMGCL	3-hydroxymethyl-3-methylglutaryl-CoA lyase
F01	NM_002130	HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
F02	NM_005518	HMGCS2	3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)
F03	NM_005357	LIPE	Lipase, hormone-sensitive
F04	NM_000237	LPL	Lipoprotein lipase
F05	NM_032601	MCEE	Methylmalonyl CoA epimerase
F06	NM_000255	MUT	Methylmalonyl CoA mutase
F07	NM_022120	OXCT2	3-oxoacid CoA transferase 2
F08	NM_018441	PECR	Peroxisomal trans-2-enoyl-CoA reductase
F09	NM_021129	PPA1	Pyrophosphatase (inorganic) 1
F10	NM_006251	PRKAA1	Protein kinase, AMP-activated, alpha 1 catalytic subunit
F11	NM_006252	PRKAA2	Protein kinase, AMP-activated, alpha 2 catalytic subunit
F12	NM_006253	PRKAB1	Protein kinase, AMP-activated, beta 1 non-catalytic subunit
G01	NM_005399	PRKAB2	Protein kinase, AMP-activated, beta 2 non-catalytic subunit
G02	NM_002730	PRKACA	Protein kinase, cAMP-dependent, catalytic, alpha
G03	NM_182948	PRKACB	Protein kinase, cAMP-dependent, catalytic, beta
G04	NM_002733	PRKAG1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit
G05	NM_016203	PRKAG2	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit
G06	NM_017431	PRKAG3	Protein kinase, AMP-activated, gamma 3 non-catalytic subunit
G07	NM_198580	SLC27A1	Solute carrier family 27 (fatty acid transporter), member 1
G08	NM_003645	SLC27A2	Solute carrier family 27 (fatty acid transporter), member 2
G09	NM_024330	SLC27A3	Solute carrier family 27 (fatty acid transporter), member 3

G10	NM_005094	SLC27A4	Solute carrier family 27 (fatty acid transporter), member 4
G11	NM_012254	SLC27A5	Solute carrier family 27 (fatty acid transporter), member 5
G12	NM_014031	SLC27A6	Solute carrier family 27 (fatty acid transporter), member 6
H01	NM_001101	ACTB	Actin, beta
H02	NM_004048	B2M	Beta-2-microglobulin
H03	NM_002046	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H04	NM_000194	HPRT1	Hypoxanthine phosphoribosyltransferase 1
H05	NM_001002	RPLP0	Ribosomal protein, large, P0
H06	SA_00105	HGDC	Human Genomic DNA Contamination
H07	SA_00104	RTC	Reverse Transcription Control
H08	SA_00104	RTC	Reverse Transcription Control
H09	SA_00104	RTC	Reverse Transcription Control
H10	SA_00103	PPC	Positive PCR Control
H11	SA_00103	PPC	Positive PCR Control
H12	SA_00103	PPC	Positive PCR Control

Supplemental Table S5: List of CT values of genes involved in fatty acid metabolism that was uploaded on to the data analysis web portal at <http://www.qiagen.com/geneglobe>

Position	Control Group	Control Group	Control Group	PG/V G Test Group 1	PG/V G Test Group 1	PG/V G Test Group 1	PG/VG+ N Test Group 2	PG/VG+ N Test Group 2	PG/VG+ N Test Group 2	PG/VG+N+ F Test Group 3	PG/VG+N+ F Test Group 3	PG/VG+N+ F Test Group 3
A01	25.455	24.70	26.12	25.91	25.70	25.38	25.24	25.12	25.79	25.82	25.69	25.68
A02	24.443	25.43	24.87	23.59	24.43	24.53	23.21	23.33	23.75	23.45	23.69	24.34
A03	26.68	36.24	26.78	25.67	26.24	26.21	25.07	24.84	25.64	25.71	25.62	25.53
A04	25.21	27.57	25.77	24.94	24.57	24.99	24.46	24.63	24.86	24.84	24.89	25.20
A05	26.45	22.39	26.23	25.83	25.89	25.63	25.29	25.33	25.54	25.71	25.70	25.79
A06	38.00	26.35	33.81	40	33.85	36.47	30.53	29.66	29.64	31.18	30.24	30.75
A07	40	35.86	38.31	34.61	35.86	32.92	28.13	28.43	28.71	30.29	31.16	31.21
A08	27.35	27.25	26.89	26.83	27.25	26.57	26.28	26.39	26.51	27.06	27.12	27.18
A09	27.27	26.50	26.78	26.31	26.50	25.87	25.71	25.84	25.70	26.59	26.46	26.71
A10	22.31	22.24	22.35	22.54	22.24	21.60	20.92	21.09	21.73	21.58	22.23	22.42
A11	25.74	24.72	25.65	25.09	24.72	24.94	23.77	23.86	25.11	24.76	25.12	25.37
A12	26.31	25.62	26.35	25.28	25.62	25.02	24.75	25.14	25.25	26.16	25.21	25.57
B01	32.18	23.13	31.57	31.27	31.13	31.29	31.20	31.14	31.20	31.64	30.71	30.95
B02	30.62	36.61	31.78	32.62	30.61	31.23	30.42	29.57	30.63	31.54	32.28	31.17
B03	34.80	32.33	35.21	39.10	34.33	34.29	34.38	34.94	33.89	34.54	34.07	33.65
B04	27.04	26.73	26.93	25.17	25.43	25.45	26.15	25.73	25.78	25.98	26.31	25.68
B05	27.12	24.91	27.29	25.94	26.91	27.08	26.00	26.53	26.54	26.32	26.42	26.71
B06	26.21	31.34	26.52	24.96	25.34	25.40	24.26	24.40	25.14	25.65	25.59	25.64
B07	26.27	25.67	26.44	25.18	25.67	25.23	24.86	24.76	25.00	25.81	25.71	26.36
B08	31.36	31.43	30.87	30.72	31.43	30.47	29.37	29.32	30.21	29.63	31.45	31.15
B09	27.56	27.19	27.76	27.34	27.19	26.94	26.93	27.16	26.68	27.89	27.44	27.38
B10	30.81	31.54	31.26	29.84	31.54	30.21	28.17	30.06	29.93	29.20	30.97	30.91
B11	27.33	27.52	28.02	27.79	27.52	27.75	28.29	28.67	28.59	28.22	28.94	28.70
B12	25.64	24.51	25.52	26.10	24.51	24.44	25.17	25.72	22.94	25.98	24.35	24.54
C01	25.35	23.74	26.25	26.12	26.74	25.46	24.14	24.76	23.95	25.86	25.55	25.07
C02	23.00	30.74	24.13	23.78	23.74	23.45	22.30	22.73	23.41	22.62	23.66	25.08
C03	28.60	28.10	28.51	31.34	29.10	28.96	28.52	28.52	28.49	28.24	28.11	28.58

C04	37.39	35.16	35.92	33.68	34.16	33.97	33.80	33.19	32.97	32.65	30.94	30.90
C05	28.58	24.69	28.61	28.97	28.69	28.31	28.31	27.99	27.51	28.45	27.95	28.58
C06	32.67	26.15	31.90	32.88	32.15	32.27	31.52	31.92	30.34	32.18	32.38	31.45
C07	29.02	29.30	29.03	29.47	29.30	29.11	29.65	29.60	29.07	30.03	29.75	29.90
C08	26.72	28.24	26.82	26.24	28.24	26.53	25.47	25.69	26.38	27.34	27.21	26.84
C09	30.86	30.85	31.23	30.35	30.85	30.48	30.15	29.51	28.22	30.92	30.39	30.01
C10	24.14	24.88	24.21	24.34	24.88	24.92	22.89	23.48	24.28	23.98	23.95	24.19
C11	26.46	26.12	26.82	26.50	26.12	26.07	24.58	24.78	26.63	25.24	26.34	26.50
C12	31.19	31.31	31.74	31.70	31.31	31.24	31.28	31.29	31.79	31.92	31.18	31.39
D01	25.49	26.92	25.57	25.21	26.92	26.15	24.60	24.66	25.39	25.50	26.25	25.77
D02	27.50	24.94	26.74	27.73	26.94	27.15	27.82	27.27	26.55	28.28	27.65	27.46
D03	26.30	32.19	25.84	24.74	25.19	24.87	24.58	24.58	24.24	24.56	24.84	24.53
D04	26.87	33.25	27.29	25.95	26.25	25.86	24.63	25.2	25.35	24.94	25.79	25.43
D05	24.27	29.44	24.92	23.90	24.44	23.25	22.41	23.10	24.36	22.71	24.84	24.89
D06	26.40	28.96	26.08	26.42	25.96	25.79	26.91	26.72	26.32	26.55	26.47	25.98
D07	24.70	24.40	24.79	23.19	24.40	23.72	23.65	23.47	22.84	24.33	24.60	24.53
D08	25.23	25.45	25.60	24.03	25.45	25.05	23.71	24.40	24.34	24.35	25.65	25.18
D09	29.29	30.39	29.75	29.49	30.39	29.53	27.24	28.26	28.18	28.26	29.52	29.25
D10	26.55	31.43	26.97	31.24	31.43	30.88	31.53	32.19	31.18	29.81	29.76	29.34
D11	33.23	34.21	32.88	34.34	34.21	34.66	33.30	34.45	39.65	32.99	37.15	36.84
D12	26.72	26.76	26.65	25.62	26.76	26.46	26.16	26.33	23.96	26.65	24.86	24.99
E01	28.90	28.43	29.36	28.72	28.43	28.67	26.64	26.68	30.76	27.21	29.90	29.50
E02	23.80	21.65	24.66	23.51	21.65	20.86	22.52	23.71	22.65	23.87	22.79	23.31
E03	30.63	29.81	29.42	30.48	29.81	29.95	30.73	30.27	29.73	30.14	29.65	29.87
E04	31.15	30.58	30.87	30.66	30.58	30.81	30.88	31.18	31.57	30.93	31.33	31.19
E05	24.93	23.67	25.37	24.31	23.67	23.32	24.12	24.09	23.46	24.41	24.40	25.28
E06	28.01	27.85	28.42	26.92	27.85	27.67	26.98	26.94	27.24	27.86	27.50	27.61
E07	34.91	34.42	34.39	35.91	34.42	32.45	31.41	32.78	32.95	32.97	34.74	34.60
E08	37.22	38.27	34.08	36.13	38.27	35.90	37.19	36.96	38.90	37.23	40	40
E09	31.69	35.65	33.30	29.17	35.65	32.66	31.95	31.85	31.77	33.41	32.98	33.72
E10	24.65	25.41	25.12	25.11	25.41	24.66	23.67	24.18	24.46	24.36	24.72	24.78
E11	24.60	24.39	24.67	23.84	24.39	24.21	23.28	23.73	23.78	24.17	24.33	24.35
E12	25.65	25.58	26.09	24.63	25.58	25.19	24.77	24.63	23.87	25.82	24.61	24.56
F01	22.74	23.07	23.40	23.15	23.07	22.69	22.78	22.80	21.90	24.10	22.56	22.33
F02	31.29	37.48	32.83	34.32	37.48	32.87	33.93	39.06	32.88	40	34.59	32.95
F03	27.67	27.73	27.39	27.60	27.73	27.43	27.59	27.33	27.25	27.74	27.74	27.66

F04	28.82	28.34	28.94	25.40	28.34	27.84	25.94	26.27	26.30	25.60	27.25	27.17
F05	28.00	27.52	28.24	27.52	27.52	27.18	26.05	26.52	26.53	26.41	27.47	27.57
F06	26.04	26.37	26.72	25.95	26.37	25.94	24.96	25.31	25.51	25.02	25.64	26.22
F07	30.48	30.67	29.12	29.74	30.67	29.58	28.59	28.44	29.25	29.23	29.42	30.28
F08	28.72	27.95	28.81	26.36	27.95	27.10	26.15	26.40	27.03	27.23	27.29	27.40
F09	23.92	24.18	24.39	22.94	24.18	23.58	22.42	23.06	22.99	23.36	23.81	23.58
F10	25.09	25.39	25.65	24.71	25.39	23.96	23.24	23.77	23.97	24.41	24.35	24.56
F11	28.49	30.09	29.92	27.47	30.09	28.71	27.56	28.34	28.08	28.54	29.33	28.91
F12	27.95	28.12	28.48	25.70	28.12	27.43	26.22	26.78	25.66	27.14	26.99	26.96
G01	26.45	27.10	27.46	26.66	27.10	27.14	25.61	26.20	25.39	25.87	26.00	26.20
G02	26.19	27.00	27.36	26.64	27.00	26.42	25.01	25.38	24.51	25.20	25.78	25.60
G03	26.70	25.88	26.08	24.79	25.88	25.79	24.62	24.93	24.72	24.70	25.17	24.86
G04	25.00	24.77	25.69	24.14	24.77	24.77	23.54	23.45	24.21	23.59	24.69	24.19
G05	27.61	27.37	27.62	26.18	27.37	27.10	25.87	26.39	25.95	26.42	26.56	26.98
G06	32.70	33.50	31.90	33.58	33.50	31.60	37.20	34.93	28.48	33.06	29.17	30.71
G07	28.39	28.21	28.16	27.42	28.21	27.75	27.50	27.64	26.91	28.42	27.85	28.51
G08	33.38	34.21	32.91	32.37	34.21	32.46	32.49	32.63	29.28	32.52	33.60	32.28
G09	28.50	28.88	28.97	27.31	28.88	28.08	27.85	27.51	27.08	28.44	28.29	28.07
G10	29.39	29.21	29.94	27.71	29.21	28.17	27.71	27.62	27.89	29.36	29.22	28.81
G11	28.11	27.69	27.75	28.07	27.69	27.41	28.32	28.24	27.94	28.55	27.59	27.87
G12	28.99	31.94	29.81	30.11	31.94	29.10	26.79	27.63	28.52	29.25	29.53	29.21
H01	19.61	19.82	20.21	18.52	19.82	18.97	18.95	18.88	17.82	19.50	19.18	18.97
H02	19.64	20.37	20.91	20.00	20.37	19.77	17.83	18.73	19.77	18.20	19.52	20.07
H03	20.97	19.78	20.17	19.61	19.78	19.19	20.01	19.23	17.95	20.16	20.12	19.39
H04	26.00	25.64	26.24	24.58	25.64	25.95	24.65	24.40	24.94	24.77	25.75	25.35
H05	20.42	19.06	20.44	18.15	19.06	18.37	18.23	17.30	17.63	17.80	18.91	18.96
H06	35.81	36.13	35.70	36.98	36.13	34.09	38.56	33.56	36.59	34.50	35.38	35.69
H07	21.89	23.77	23.15	21.52	23.77	23.15	24.99	22.83	22.82	23.57	22.54	24.70
H08	21.52	23.80	23.74	22.38	23.80	23.10	22.09	21.68	23.44	22.32	24.29	24.91
H09	20.87	24.34	23.44	20.48	24.34	23.17	20.62	21.13	23.24	21.19	23.09	23.90
H10	28.09	29.83	28.24	27.31	29.83	28.69	26.78	27.74	28.05	28.14	28.86	28.56
H11	27.68	28.87	29.02	27.21	28.87	27.76	26.82	28.89	28.11	28.13	28.78	28.57
H12	27.96	29.08	28.18	27.46	29.08	29.16	26.52	27.79	26.73	27.91	28.55	28.554

Supplemental Table S6: Fold regulation and p-Values of genes associated with fatty acid metabolism analyzed in the study.

Fold Regulation (comparing to control group)

Position	Gene Symbol	Group 1		Group 2		Group 3	
		Fold Regulation	p-Value	Fold Regulation	p-Value	Fold Regulation	p-Value
A01	ACAA1	-2.01	0.048542	-2.8	0.016388	-2.21	0.027894
A02	ACAA2	-1.03	0.776242	-1.03	0.792344	1.18	0.714739
A03	ACAD10	6.4	0.6315	6.82	0.524766	8.11	0.318698
A04	ACAD11	1.5	0.70867	1	0.607066	1.29	0.974287
A05	ACAD9	-2.88	0.344697	-3.72	0.322925	-2.93	0.342622
A06	ACADL	-12.68	0.369737	1.18	0.416386	1.11	0.417011
A07	ACADM	1.04	0.708738	32.85	0.006002	9.59	0.000668
A08	ACADS	-1.4	0.251221	-1.7	0.134504	-1.74	0.125277
A09	ACADSB	-1.11	0.649403	-1.35	0.168952	-1.5	0.139147
A10	ACADVL	-1.51	0.191533	-1.39	0.257033	-1.53	0.043931
A11	ACAT1	-1.24	0.5143	-1.33	0.557376	-1.47	0.071616
A12	ACAT2	1.02	0.859647	-1.4	0.137779	-1.31	0.524461
B01	ACOT1	-8.21	0.373023	-13.43	0.371535	-7.89	0.373397
B02	ACOT12	1.16	0.752789	1.66	0.866835	-1.03	0.429374
B03	ACOT6	-2.4	0.280458	-3.71	0.201734	-1.86	0.355934
B04	ACOT7	1.72	0.035566	-1.43	0.045815	1.05	0.767286
B05	ACOT8	-1.96	0.280723	-2.73	0.203106	-1.85	0.2926
B06	ACOT9	4.06	0.24632	3.71	0.354409	2.93	0.658994
B07	ACOX1	-1	0.99261	-1.22	0.111804	-1.6	0.04958
B08	ACOX2	-1.34	0.340069	1.04	0.927867	-1.29	0.561817
B09	ACOX3	-1.34	0.169607	-1.94	0.000816	-1.88	0.019001
B10	ACSBG1	-1.07	0.796352	1.22	0.574991	1	0.941453
B11	ACSBG2	-1.78	0.077905	-5.38	0.003044	-3.58	0.004257
B12	ACSL1	-1.48	0.607042	-1.89	0.535272	-1.49	0.552809
C01	ACSL3	-3.39	0.132828	-1.63	0.318414	-2.33	0.201804
C02	ACSL4	2.89	0.642993	3.05	0.750924	2.51	0.651126
C03	ACSL5	-4.49	0.009226	-3.12	0.000959	-1.68	0.035302

C04	ACSL6	1.22	0.250416	1.1	0.600237	6.3	0.073107
C05	ACSM3	-4.38	0.312885	-4.52	0.308799	-3.68	0.325518
C06	ACSM4	-7.8	0.359158	-5.88	0.363639	-6.1	0.362462
C07	ACSM5	-1.93	0.074261	-3.62	0.015483	-3.07	0.022552
C08	ALDH2	-1.43	0.374609	-1.08	0.667734	-1.64	0.252543
C09	BDH1	-1.27	0.067749	1.12	0.609952	-1.23	0.609427
C10	BDH2	-2.1	0.088246	-1.59	0.339097	-1.39	0.282793
C11	CPT1A	-1.45	0.160809	-1.31	0.862862	-1.32	0.160847
C12	CPT1B	-1.71	0.08074	-2.98	0.005024	-1.9	0.062988
D01	CPT1C	-1.82	0.207438	-1.34	0.403861	-1.61	0.227743
D02	CPT2	-3.14	0.168202	-5.11	0.11551	-4.74	0.122737
D03	CRAT	5.33	0.288872	4.32	0.550669	6.17	0.172267
D04	CROT	5.09	0.292138	5.71	0.248712	7.48	0.053914
D05	DECR1	2.98	0.60495	2.62	0.662377	2.33	0.838443
D06	DECR2	1.25	0.818553	-2.05	0.213949	-1.02	0.532141
D07	ECHS1	1.07	0.550888	-1.16	0.346152	-1.62	0.005287
D08	ECI2	-1.14	0.593853	-1.2	0.594086	-1.39	0.152046
D09	EHHADH	-1.7	0.162567	1.31	0.572161	-1.03	0.754397
D10	FABP1	-12.41	0.116284	-28.9	0.11077	-4.48	0.136404
D11	FABP2	-3.32	0.103371	-5.06	0.085761	-3.32	0.11947
D12	FABP3	-1.26	0.289409	-1.24	0.975776	1.29	0.390821
E01	FABP4	-1.39	0.235217	-1.58	0.773429	-1.76	0.452171
E02	FABP5	1.51	0.50594	-2.18	0.301157	-1.74	0.399356
E03	FABP6	-1.86	0.250142	-3.53	0.083747	-1.71	0.262328
E04	FABP7	-1.5	0.143765	-3.67	0.004842	-2.18	0.009357
E05	FASN	1.09	0.758268	-1.7	0.152533	-1.84	0.155805
E06	GCDH	-1.12	0.364784	-1.41	0.039111	-1.33	0.223062
E07	GK	-1.11	0.792326	1.58	0.368644	-1.3	0.554187
E08	GK2	-2.1	0.186509	-3.57	0.09803	-2.22	0.168222
E09	GPD1	1.22	0.595872	-1.04	0.519851	-1.84	0.319307
E10	GPD2	-1.7	0.131449	-1.48	0.283446	-1.32	0.263873
E11	HADHA	-1.28	0.069132	-1.49	0.103464	-1.49	0.027429
E12	HMGCL	-1.09	0.373878	-1.13	0.553173	-1.05	0.794041

F01	HMGCS1	-1.59	0.112293	-1.94	0.047888	-1.7	0.362526
F02	HMGCS2	-3.45	0.230846	-5.37	0.195291	-3.95	0.227631
F03	LIPE	-1.69	0.119489	-2.51	0.037891	-1.94	0.078766
F04	LPL	1.67	0.369455	1.99	0.029435	2.27	0.094906
F05	MCEE	-1.2	0.326859	1.01	0.827637	-1.05	0.828492
F06	MUT	-1.39	0.131583	-1.33	0.30076	-1.06	0.801114
F07	OXCT2	-1.59	0.369865	-1.15	0.616168	-1.32	0.50045
F08	PECR	1.51	0.115709	1.36	0.267124	1.27	0.154291
F09	PPA1	-1.13	0.492561	-1.14	0.69016	-1.2	0.252519
F10	PRKAA1	-1.06	0.941731	1.13	0.603804	1.06	0.766058
F11	PRKAA2	-1.02	0.943831	-1.01	0.805619	-1.21	0.505089
F12	PRKAB1	1.26	0.496432	1.35	0.255236	1.24	0.30915
G01	PRKAB2	-1.67	0.147342	-1.2	0.524918	1.1	0.82888
G02	PRKACA	-1.52	0.238998	1.28	0.522283	1.39	0.318521
G03	PRKACB	-1.02	0.887202	-1.05	0.776717	1.38	0.152692
G04	PRKAG1	-1.13	0.401189	-1.08	0.846456	1.11	0.465316
G05	PRKAG2	-1.09	0.663927	-1.05	0.852306	1.03	0.838065
G06	PRKAG3	-1.95	0.373751	-3.1	0.710624	1.84	0.372498
G07	SLC27A1	-1.24	0.239276	-1.55	0.105537	-1.8	0.112541
G08	SLC27A2	-1.21	0.576657	1.42	0.51309	-1.11	0.692374
G09	SLC27A3	-1.05	0.835354	-1.17	0.40721	-1.25	0.387841
G10	SLC27A4	1.3	0.17472	1.18	0.21304	-1.38	0.188936
G11	SLC27A5	-1.56	0.176814	-3.6	0.008332	-1.99	0.096795
G12	SLC27A6	-1.87	0.327645	2.1	0.434841	1.05	0.579324
H01	ACTB	1.01	0.988641	-1.15	0.684315	-1.13	0.692316
H02	B2M	-1.42	0.292702	-1	0.815027	1.15	0.712705
H03	GAPDH	1.01	0.952745	-1.22	0.796888	-1.34	0.41314
H04	HPRT1	-1.15	0.67802	-1.17	0.31048	-1.12	0.193351
H05	RPLP0	1.61	0.071266	1.65	0.123996	1.49	0.20647