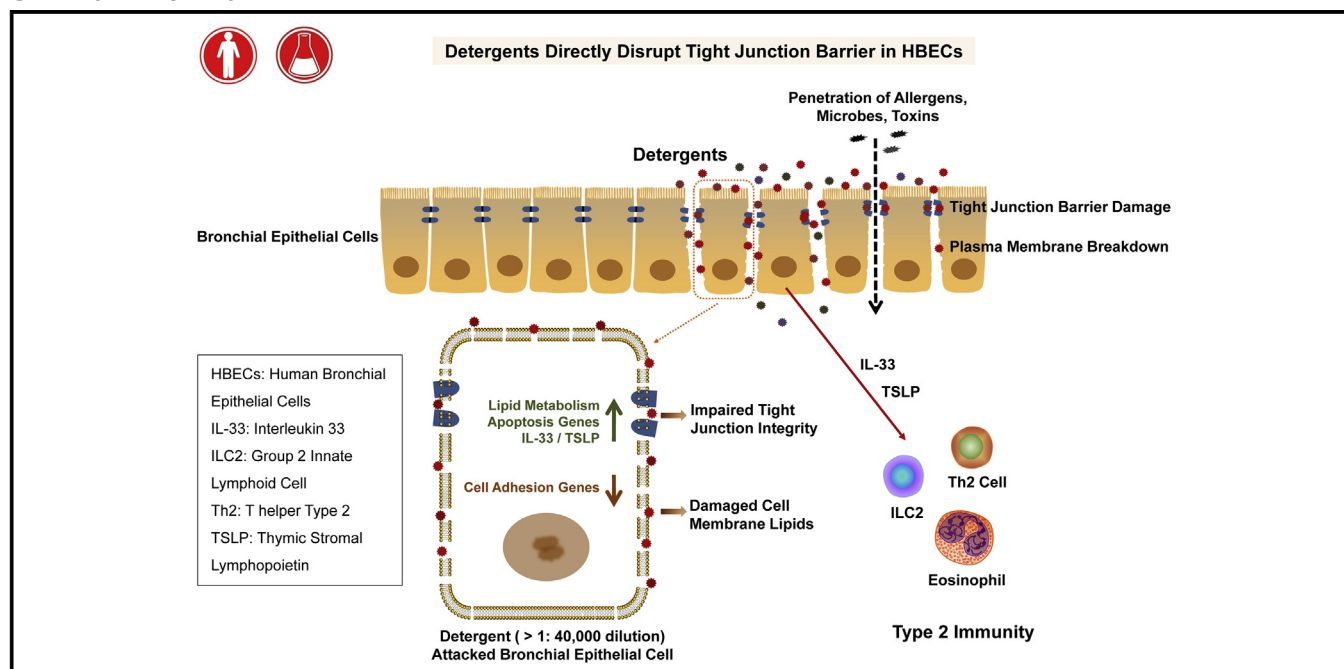


Laundry detergents and detergent residue after rinsing directly disrupt tight junction barrier integrity in human bronchial epithelial cells



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GRAPHICAL ABSTRACT



Background: Defects in the epithelial barrier have recently been associated with asthma and other allergies. The influence of laundry detergents on human bronchial epithelial cells (HBECS) and their barrier function remain unknown.

Objective: We investigated the effects of laundry detergents on cytotoxicity, barrier function, the transcriptome, and the epigenome in HBECS.

Methods: Air-liquid interface cultures of primary HBECS from healthy control subjects, patients with asthma, and patients with chronic obstructive pulmonary disease were exposed to laundry detergents and detergent residue after rinsing. Cytotoxicity and epithelial barrier function were evaluated. RNA sequencing, Assay for Transposase Accessible Chromatin with high-throughput sequencing, and DNA

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Disclosure of potential conflict of interest: G. Tan, P. Wawrzyniak, P. Westermann, and A. Dreher are employed by the Swiss Institute of Allergy and Asthma Research. C. A. Akdis has consultant arrangements with Actellion, Aventis, Stallergenes, Allergopharma, and Circacia; is employed by the Swiss Institute of Allergy and Asthma Research; and has received grants from Novartis, PREDICTA: European Commission's Seventh Framework programme no. 260895, the Swiss National Science

Foundation, MeDall: European Commission's Seventh Framework Programme no. 261357, and the Christine Kühne-Center for Allergy Research and Education. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication June 25, 2018; revised October 31, 2018; accepted for publication November 9, 2018.

Available online November 27, 2018.

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0091-6749/\$36.00

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<https://doi.org/10.1016/j.jaci.2018.11.016>

methylation arrays were used for checking the transcriptome and epigenome.

Results: Laundry detergents and rinse residue showed dose-dependent toxic effects on HBECs, with irregular cell shape and leakage of lactate dehydrogenase after 24 hours of exposure. A disrupted epithelial barrier function was found with decreased transepithelial electrical resistance, increased paracellular flux, and stratified tight junction (TJ) immunostaining in HBECs exposed to laundry detergent at 1:25,000 dilutions or rinse residue at further 1:10 dilutions. RNA sequencing analysis showed that lipid metabolism, apoptosis progress, and epithelially derived alarmin-related gene expression were upregulated, whereas cell adhesion-related gene expression was downregulated by laundry detergent at 1:50,000 dilutions after 24 hours of exposure without substantially affecting chromatin accessibility and DNA methylation.

Conclusion: Our data demonstrate that laundry detergents, even at a very high dilution, and rinse residue show significant cell-toxic and directly disruptive effects on the TJ barrier integrity of HBECs without affecting the epigenome and TJ gene expression. (J Allergy Clin Immunol 2019;143:1892-903.)

Key words: Laundry detergent, rinse residue, barrier function, tight junction, bronchial epithelial cells, asthma, chronic obstructive pulmonary disease, transcriptome, epigenome

The prevalence of asthma and allergic diseases has markedly increased worldwide since the 1960s.¹ Compared with 50 years ago, the incidence of asthma has increased by 2- to 3-fold in children.² It is still unclear why this increase is occurring, but it is generally accepted that asthma and allergies are associated with the environment of a modern lifestyle.^{3,4} It is well documented that increased exposure to multiple environmental factors, such as air pollutants, tobacco smoke, allergens in the air, fragrances, and preservatives, contribute to the development and exacerbation of asthma and other allergies.^{5,6} There is growing evidence demonstrating that subjects repeatedly and highly exposed to cleaning agents both in the workplace and at home were at high risk of allergen sensitization and asthma,⁷⁻¹⁰ suggesting an important public health issue.

The popularizing of synthetic laundry detergents coincided with the uprising of allergic diseases over the past few decades. Laundry detergents are produced in large quantities and used in various daily life and industrial activities. As one of the substances with the most common exposure in the human living environment, laundry detergents, especially the detergent rinse residue remaining in clothing after laundry, could have a high likelihood of being inhaled into the airways and reaching beneath the upper and lower respiratory mucosae.

Laundry detergents contain numerous ingredients that might be irritants (surfactants and bleaches) or sensitizers (fragrances and enzymes) and can cause asthma and other allergic diseases.⁵ Asthma symptoms in domestic cleaning workers are associated with exposure to bleaches and other irritant agents in detergent.¹¹ Several studies also reported occupational asthma in detergent factories.¹²⁻¹⁴

Airway epithelial cells form a surface barrier against inhaled environmental insults. Increasing evidence indicates that defective epithelial barrier function is associated with asthma.^{15,16} It

Abbreviations used

ALI:	Air-liquid interface
ATAC-seq:	Assay for Transposase Accessible Chromatin with high-throughput sequencing
BP:	Biological process
COPD:	Chronic obstructive pulmonary disease
DEG:	Differentially expressed gene
DMP:	Differentially methylated probe
FITC:	Fluorescein isothiocyanate
GO:	Gene Ontology
HBEC:	Human bronchial epithelial cell
LDH:	Lactate dehydrogenase
RNA-seq:	RNA sequencing
SDBS:	Sodium dodecyl benzene sulfonate
TER:	Transepithelial electrical resistance
TJ:	Tight junction
TSLP:	Thymic stromal lymphopoietin
UPLC:	Ultraperformance liquid chromatography
ZO-1:	Zonula occludens 1

was hypothesized that the allergenicity of inhaled substances depends on their potential to disrupt epithelial barriers.¹⁷ We have previously demonstrated that anionic surfactants, one of the main constituents of detergents, cosmetics, and toothpastes, disrupted the barrier integrity of human keratinocytes,¹⁸ which suggests that the possibility of sensitization caused by chronic exposure of certain detergents should be taken into consideration.

This study aims to investigate the effects of laundry detergents on epithelial barrier function and on the transcriptome and epigenome in human bronchial epithelial cells (HBECs). We demonstrated that 1-time exposure of HBECs to laundry detergents and rinse residue led to a direct tight junction (TJ)-attacking and barrier-disruptive effect on the integrity of HBECs without affecting DNA methylation and chromatin accessibility. Changes in RNA sequencing (RNA-seq) indicate a downregulation of cell adhesion-related genes, and upregulated lipid metabolism and apoptosis-related genes can be associated with impaired barrier function at air-liquid interface (ALI)-cultured HBECs.

METHODS

Cell cultures

Primary HBECs of healthy control subjects, asthmatic patients, and patients with chronic obstructive pulmonary disease (COPD) were purchased from Epithelix (Geneva, Switzerland). Cells were cultured as monolayers in bronchial epithelial basal medium (Lonza, Basel, Switzerland) supplemented with the SingleQuot Kit placed in a 75-cm² T-flask in a humidified incubator at 37°C in 5% CO₂. HBECs in passage 2 or 3 were seeded in ALI cultures onto 6.5-mm-diameter polyester membrane transwells with a pore size of 0.4 μm (growth area, 0.33 cm²; COSTAR; Corning, Corning, NY) at a density of 150,000 cells per well. Bronchial epithelial growth medium supplemented with the SingleQuot kit, except for retinoic acid and triiodothyronine (Lonza), was mixed 1:1 with Dulbecco modified Eagle medium (Gibco-BRL, Invitrogen, Carlsbad, Calif). Fresh all-trans retinoic acid (Sigma-Aldrich, Saint Louis, Mo) was supplemented at a concentration of 10 ng/mL. Medium was changed every second day. Cells were grown submerged for 4 days to complete confluence, apical medium was removed, and bronchial epithelial growth medium–Dulbecco modified Eagle medium at a ratio of 1:1 with all-trans retinoic acid was added basolaterally to differentiate these cells. Epithelial cell cultures were used for *in vitro* detergent exposure studies on day 21 at the ALI.

Rinse residual liquid collection of detergents and concentration measurement

Normal laundry was performed to collect detergent rinse residual liquid. For cleaning the laundry machine (WA 2352; Bauknecht, Stuttgart, Germany), a washing program was run 1 time using an easy-care model with commercial detergent A. Four kilograms of clean bedclothes and a recommended amount of detergent A (73 mL) were used for collecting the residual liquid. The washing program was an easy-care model with a spin speed of 600 revolutions per minute and water temperature of 40°C. Total water consumption of 1-time laundry was 63.5 L, of which 23.8 L of water was for the washing cycle, 35.2 L of water was for the rinse cycle, and 4.5 L of water remained in clothing. The rinse residue liquid was collected from wet clothes and sterilized by passing through a 0.22- μ m filter.

The concentration of sodium dodecyl benzene sulfonate (SDBS) in rinse residual liquid was measured with the polar C18 column by using ultra-performance liquid chromatography (UPLC; ACQUITY UPLC H-Class Bio System; Waters Corporation, Milford, Mass). Serial dilutions of SDBS were used to make the standard curve. Detergent A was diluted 1:2500 as a control. The total rinse factor was calculated according to consumed water at the washing cycle divided by the amount of detergent and multiplied by consumed water at the rinse cycle divided by remaining water in clothing and together with the concentration of SDBS in residual liquid was compared with original detergent A liquid. Based on these 2 results, the rinse factor was approximately 1:2500 in this study.

Detergent exposures

Detergent A or rinse residue was diluted in the above-mentioned ALI culture medium (vol/vol). ALI cultures on day 21 were exposed to different detergent or rinse residue doses by adding to the apical compartment for different time points, as indicated in figures and their legends. Detergent A was used at concentrations of 1:50,000 and 1:25,000 (vol/vol). Concentrations of rinse residue for exposure were 1:10 and 1:5 (vol/vol). For the unexposed control group, only medium was added to the apical compartment.

Cytotoxicity measurement

For monolayer cultures, cells were treated for 24 hours with commercial detergent A or B at concentrations of $1:0.5 \times 10^4$, $1:2 \times 10^4$, $1:4 \times 10^4$, $1:8 \times 10^4$, and $1:16 \times 10^4$ (vol/vol). For ALI cultures, cells were exposed to commercial detergent A at concentrations of 1:50,000 and 1:25,000 (vol/vol) or rinse residue at concentrations of 1:10 and 1:5 (vol/vol) for 72 hours. Cytotoxicity was evaluated by measuring the amount of lactate dehydrogenase (LDH) released into culture medium at 72 hours after exposure to detergents or detergent residue after rinse by using the Pierce LDH Cytotoxicity Assay Kit (Thermo Scientific, Rockford, Ill). Briefly, 50 μ L of medium from each culture was transferred to a 96-well plate and mixed with 50 μ L of reaction mixture before incubation at room temperature for 30 minutes in the dark. At the end of incubation, absorbance was measured at 492 nm and 620 nm as a reference wavelength. Cytotoxicity measurements were performed in duplicate samples, and LDH values were calculated, as suggested by the manufacturer's instructions, including a background control for LDH activity in the media, a control for untreated cells (0% lysis of the cells), and a control for maximum LDH release (100% lysis of the cells). Phase-contrast images were taken for monolayer cultures just before cytotoxicity measurement.

Transepithelial electrical resistance and paracellular flux measurement

Transepithelial electrical resistance (TER) measurements were done at 0, 24, 48, and 72 hours after detergent exposure by using a Millicell-ERS Volt Ohm Meter (Millipore, Temecula, Calif). Samples from each ALI culture donor were measured in triplicates in multiple-well systems. Wells not building up (that did not reach confluence) sufficiently (TER >400 $\Omega \times \text{cm}^2$) were not included in experiments.

Paracellular flux measurements were performed after TER experiments. Paracellular permeability was evaluated 24 hours after apical addition of 2 mg/mL fluorescein isothiocyanate (FITC)-dextran based on the intensity of FITC in the basolateral medium, as determined with an ELISA reader (Mithra LB 940; Berthold Technologies, Bad Wildbad, Germany) at 480 nm. Each donor culture was measured in a minimum of duplicates in multiple-well systems.

Immunofluorescence staining

ALI cultures were fixed with 4% paraformaldehyde (Fluka, St Louis, Mo) 72 hours after detergent exposure. ALI samples were blocked with goat serum in 1% BSA/PBS and incubated with Alexa Fluor 488-labeled anti-occludin mAb (Invitrogen) and anti-zonula occludens 1 (ZO-1)-unlabeled antibody (Invitrogen) and then detected with Alexa Fluor 546-labeled goat anti-rabbit secondary antibody (Invitrogen). Samples were counterstained with 4,6-diamidino-2-phenylindole for nuclear staining and mounted with ProLong-Gold containing antifade reagent (Invitrogen). Specimens were examined under a Zeiss LSM 780 confocal microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany).

RNA isolation, RNA-seq, and data analysis

ALI cultured HBECs from healthy control subjects, asthmatic patients, and patients with COPD were harvested 24 hours after laundry detergent A exposure at a concentration of 1:50,000. Total RNA was extracted and purified with an RNeasy Plus Micro Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The quantity and quality of the isolated RNA were determined with the NanoDrop 2000 Spectrophotometer (Thermo Fischer Scientific) and 2200 TapeStation Automated Electrophoresis System (Agilent Technologies, Santa Clara, Calif), and samples with an RNA integrity number of greater than 9.0 were chosen for sequencing. Library preparation for RNA-seq was performed with the TruSeq Stranded mRNA Sample Prep Kit (Illumina, San Diego, Calif). Sequencing was performed on the Illumina HiSeq 4000.

Adapters and low-quality tails were trimmed from reads before read alignment. STAR aligner (version 2.5.3a)¹⁹ was used to align the RNA-seq data to Ensembl reference genome build GRCh38.p10. Gene expression values were quantified by feature Counts from the Bioconductor package Rsubread (version 1.28.0).²⁰ A gene was considered expressed if, in at least 1 group of comparison, it had more than 10 counts in more than half of the replicates. Differentially expressed genes (DEGs) were assessed with a generalized linear model with the quasiliikelihood test from the Bioconductor package edgeR (version 3.20.1).²¹ Genes with a *P* value of less than .01 and log₂ fold change of greater than 0.5 or less than -0.5 were included in this study. Overrepresentation of Gene Ontology (GO) categories was performed by using the Bioconductor package goseq (version 1.28.0)²² with the Wallenius approximation.

Assay for Transposase Accessible Chromatin with high-throughput sequencing and data analysis

After 24 hours of exposure to detergent A at a concentration of 1:50,000, ALI-cultured HBECs from healthy control subjects, asthmatic patients, and patients with COPD were harvested and immediately continued to lysis and transposition reactions.²³ Briefly, cell lysates were incubated with the Nextera Tn5 Transposase (Illumina) at 37°C for 30 minutes. Immediately after transposition, DNA was purified with a Qiagen MinElute PCR Purification Kit. Libraries were generated with Ad1_noMX and Ad2.1-2.4 barcoded primers and purified, as previously described.²⁴ Library quality was assessed by using the Agilent Genomic DNA Screen Tape System. Sequencing was performed on the Illumina HiSeq 2500.

Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq) reads were processed by the processing pipeline, which meets ENCODE official pipeline specification. For more details, please refer to https://github.com/kundajelab/atac_dnase_pipelines. It includes quality control, producing alignments, producing peaks, and signal tracks and enrichment analysis. Promoter regions that were differentially accessible

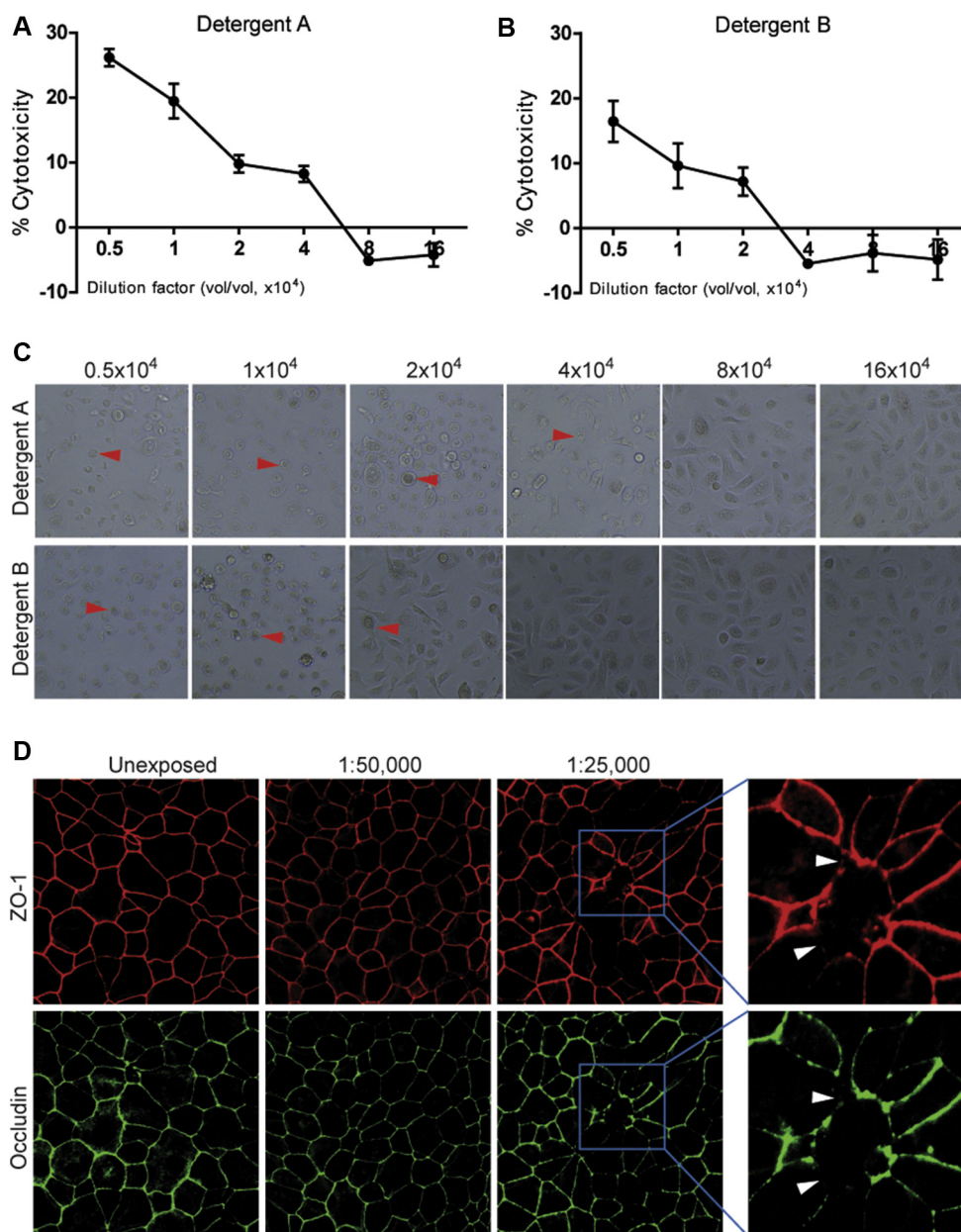


FIG 1. Dose-dependent cytotoxicity and disruption of barrier integrity in HBEs in response to laundry detergents. **A** and **B**, Cytotoxicity of monolayer-cultured HBEs treated with commercial detergents A and B for 24 hours at different dilutions. Data are presented as means $\pm$ SDs ($n = 3$ per group in duplicate cultures). **C**, Representative phase-contrast images of monolayer-cultured HBEs treated with detergent A or detergent B for 24 hours. Dilution factors are labeled at the top. **D**, Immunofluorescence staining of ZO-1 (red) and occludin (green) in ALI-cultured HBEs exposed to detergent A for 72 hours at concentrations of 1:50,000 (vol/vol) and 1:25,000 (vol/vol). One representative of 5 different stainings is shown.

between the unexposed and detergent-exposed groups were identified based on $\log_2$ fold change of greater than 0.5 or less than -0.5 plus a significant P value of less than .01.

Methylation EPIC array and data analysis

Genomic DNA was isolated from detergent A-treated ALI cultures from healthy control subjects and asthmatic patients. Bisulfite modification was then carried out by using Direct Bisulfite Treatment based on a Zymo Research (Irvine, Calif)-recommended protocol with modification. The human FKBP5 ADS6607 DNA methylation assay was selected to assess the

quality of bisulfite-converted DNA. By using this method, the DNA samples were determined to contain DNA that can be amplified and bisulfite sequenced. DNA methylation across the genome were measured by using Infinium Methylation EPIC BeadChips (EpigenDX, Hopkinton, Mass; including $>850,000$ methylation sites), according to the manufacturer's instructions. The methylation EPIC array was processed and analyzed by using standard Bioconductor workflow.²⁵ In brief, unwanted variation within and between groups was minimized by using quantile normalization. Before differential methylation analysis, probes affected by single nucleotide polymorphisms and low-quality probes with detection P values of less than .01 in at least 1 sample were removed. Then we used Bioconductor

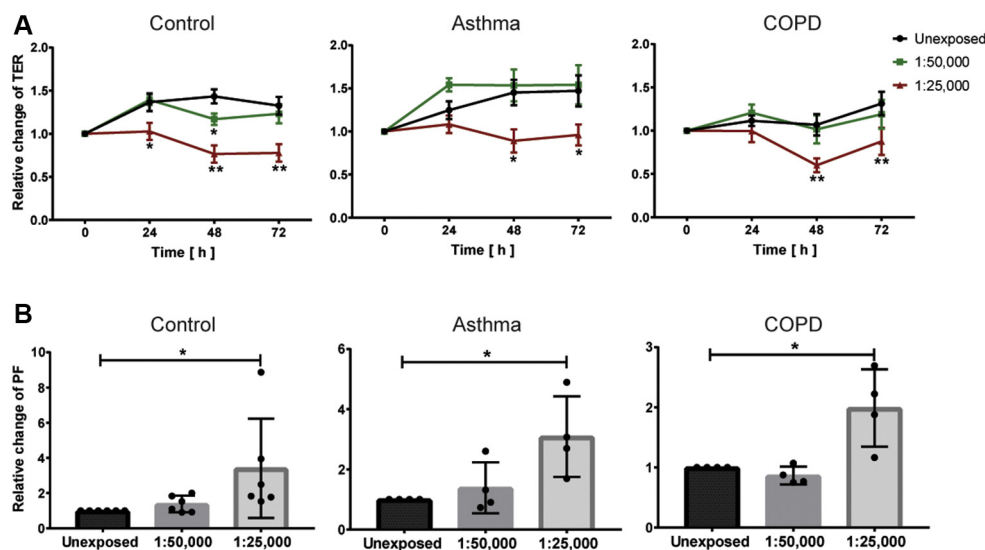


FIG 2. Laundry detergent A decreased TER and increased paracellular flux in ALI-cultured HBECs. **A**, TER was measured at 0, 24, 48, and 72 hours after detergent A exposure at concentrations of 1:50,000 and 1:25,000 in ALI-cultured HBECs from healthy control subjects, asthmatic patients, and patients with COPD. **B**, Paracellular flux (PF) in response to 72 hours of exposure to detergent A in ALI-cultured HBECs from healthy control subjects, asthmatic patients, and patients with COPD. Data are presented as means $\pm$ SDs ($n = 5$ for the control group, $n = 4$ for asthma group, and $n = 4$ for the COPD group in triplicates). * $P < .05$ and ** $P < .01$, Wilcoxon matched-pairs test.

package limma (version 3.34.9)²⁶ to perform probe-wise differential methylation analysis.

Statistical analysis

Data analysis was performed with GraphPad Prism software (version 7.0; GraphPad Software, La Jolla, Calif). Differences between paired groups were computed by using the Wilcoxon signed-rank test. For unmatched groups, differences were evaluated by using the Mann-Whitney test. Differences were considered significant at a P value of less than .05.

RESULTS

Laundry detergents cause cytotoxicity and impaired barrier integrity in HBECs

To investigate the effects of commonly used laundry detergents on HBECs, we first assessed their cytotoxic effects. Serially diluted commercial laundry detergents were added into monolayer-cultured HBECs. Cytotoxicity was determined by measuring the amount of LDH released into the culture medium. Dose-dependent cytotoxicity was found in monolayer-cultured HBECs responding to both detergent A and detergent B treatment. Starting from the dilution of 1:40,000 of commercial detergent A and 1:20,000 dilution of detergent B, cells showed lysis in response to detergent exposure (Fig 1, A and B). Cells observed by using phase-contrast microscopy showed irregular morphology with round and shrinking shapes, detachment from the plate, and breakdown of cytosolic membrane compared with control cells (Fig 1, C).

We further developed ALI cultures of HBECs and exposed them to detergents at concentrations of 1:50,000 and 1:25,000. Immunofluorescence staining of the TJ proteins occludin and ZO-1 were then used to assess barrier integrity. As shown in Fig 1, D, exposure to detergent A (1:25,000) led to a marked disruption

of TJ integrity with irregular, opened, and patchy immunostaining of occludin and ZO-1, whereas immunostaining in the control group revealed intact TJ integrity.

Laundry detergents disrupt barrier function of ALI-cultured HBECs

After detergent exposure, TER and paracellular diffusion of FITC-labeled 4-kDa dextran were detected as surrogate markers of barrier function in ALI-cultured HBECs from healthy control subjects, asthmatic patients, and patients with COPD. A significantly lower TER was observed 48 and 72 hours after detergent exposure at a concentration of 1:25,000 compared with the unexposed group in ALI-cultured HBECs (Fig 2, A). Measurement of paracellular flux was significantly increased with detergent A exposure at a concentration of 1:25,000 in parallel ALI cultures of HBECs from healthy control subjects, asthmatic patients, and patients with COPD, confirming the TER results (Fig 2, B).

Detergent residue after rinsing show toxicity on HBECs

Rinse residue in clothing has a very high chance of inhalation into the airways and direct contact with the skin and accessible mucosa. To assess the quantity of detergent residue after a full rinse, we collected residual liquid from wet clothes immediately after laundry and measured the concentration of SDBS, one of the main surfactant ingredients of detergent A, using UPLC. The concentration of carbon 10 SDBS in residual liquid (0.049 mg/mL) was almost the same amount of SDBS in detergent A at 1:2500 dilution (0.052 mg/mL; Fig 3, A).

We then measured the level of protease (a typical detergent enzyme) in newly washed and dried clothing samples. Residual

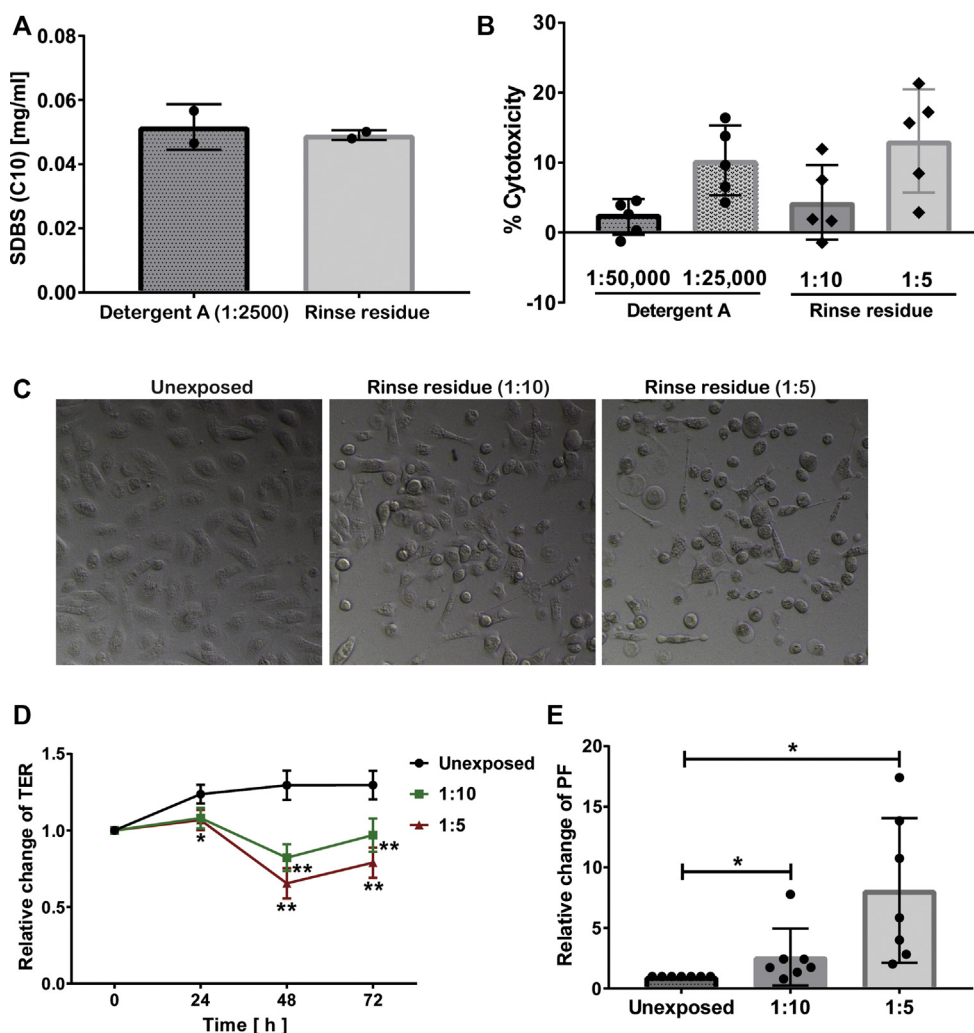


FIG 3. Detergent residue after rinsing disrupted barrier function in HBECs. **A**, Concentration of carbon 10 SDBS in detergent A rinse residual liquid was measured by using UPLC. Detergent A was diluted at 1:2500 for comparison. **B**, Cytotoxicity of ALI-cultured HBECs exposed to detergent A or rinse residue at different concentrations. Data are presented as means $\pm$ SDs ($n = 5$ for each group in triplicates). **C**, Representative phase-contrast images of monolayer-cultured HBECs treated with rinse residue at concentrations of 1:10 and 1:5. **D** and **E**, Rinse residue at concentrations of 1:10 and 1:5 decreased TER and increased paracellular flux in ALI-cultured HBECs from healthy control subjects. Data are presented as means $\pm$ SDs ($n = 7$ per group in triplicates). * $P < .05$ and ** $P < .01$, Wilcoxon matched-pairs test.

protease levels in clothing with different fabrics were 5.4, 5.2, and 4.2 mU/g for cotton, polyester, and wool, respectively, which also indicated a large amount of residual enzyme in clothing compared with protease levels in original laundry detergent liquid (36.3 mU/g, see Fig E1 in this article's Online Repository at www.jacionline.org).

Furthermore, we measured the toxic effect of residual liquid on ALI-cultured HBECs. The result of LDH activity showed that residual liquid diluted at both 1:10 and 1:5 was toxic to cells. Similar results were found in detergent A diluted at 1:50,000 and 1:25,000 (Fig 3, B). Rinse residue was also added to monolayer-cultured HBECs for 24 hours to observe cell morphology. The phase-contrast images indicated similar changes in cell morphology, as described above for detergent exposure (Fig 3, C).

Detergent residue after rinsing disrupt barrier function of HBECs

TER and paracellular flux were measured after exposure to rinse residue at concentrations of 1:10 and 1:5 dilution to investigate the effect of detergent residue after rinse on barrier function of ALI-cultured HBECs. TER was significantly decreased by rinse residue after 24 hours of exposure at both concentrations (Fig 3, D). In addition, paracellular flux was significantly increased in HBECs after 72 hours of exposure to rinse residue at concentrations of both 1:10 and 1:5, confirming the TER results (Fig 3, E).

Immunofluorescence staining of occludin and ZO-1 was then performed to measure TJ integrity. Irregular and opened staining of occludin and ZO-1 was found in ALI-cultured HBECs exposed to rinse residue at 1:10 dilution. Furthermore, severe disruption

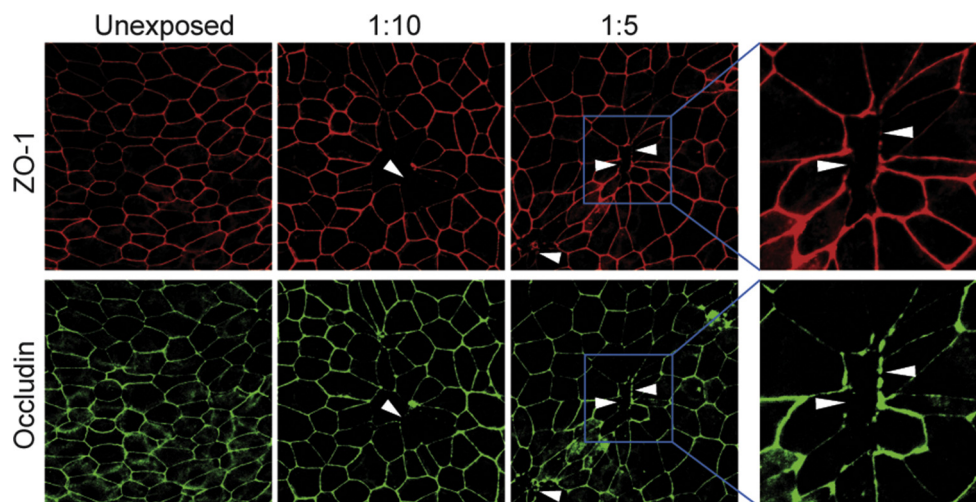


FIG 4. Detergent residue after rinsing impaired TJ integrity of HBECs. Representative immunofluorescence staining of ZO-1 (red) and occludin (green) in ALI-cultured HBECs from healthy control subjects exposed for 72 hours or unexposed to rinse residue at concentrations of 1:10 and 1:5. One representative of 5 different stainings is shown.

with patchy and broken TJ staining was shown in cells exposed to a higher dose of rinse residue (1:5 dilution), which indicated impairment of barrier integrity (Fig 4). These results obtained from rinse residue are in agreement with those obtained from laundry detergent, confirming the effects of detergent on HBECs.

Reagent was used for cell exposure to exclude possible contamination of endotoxin in detergent. Endotoxin assays were performed in rinse residue and different dilutions of laundry detergent. The results showed that there is no endotoxin in all serial dilutions of detergent (1:2,500, 1:25,000, and 1:50,000). Similarly, there are trace amounts of endotoxin in rinse residue (0.01 ng/mL), which is far lower than values in the concentration-disrupted epithelial barrier (0.3 ng/mL, see Fig E2 in this article's Online Repository at www.jacionline.org).²⁷ Therefore we excluded the endotoxin influence on barrier function.

Negligible effect of laundry detergents on chromatin accessibility and DNA methylation

The epigenome can be viewed as an interface between the environment and the genome and also reshapes the transcriptome,²⁸ and recent studies have demonstrated epigenetic mechanisms that are associated with allergic inflammation.^{29,30} Accordingly, we performed DNA Methylation Chip analysis and ATAC-seq experiments on ALI-cultured HBECs to investigate the effect of laundry detergent on epigenome. The ATAC-seq results showed that approximately 23% of the putative chromatin accessible regions were located in the promoter region (Fig 5, A, and see Fig E3, A and B, in this article's Online Repository at www.jacionline.org). In addition, as expected, there is strong ATAC-seq read enrichment around the transcriptional start site, which is consistent with these regions containing *cis*-regulatory elements (Fig 5, B, and see Fig E3, C, in this article's Online Repository at www.jacionline.org).

The Spearman correlation coefficient was calculated between all samples from the control, asthma, and COPD groups based on read coverage to assess the influence of detergents on chromatin accessibility. The result showed no clear separation between

detergent-exposed and unexposed samples, which indicated no effect of the detergent on chromatin accessibility (see Fig E4 in this article's Online Repository at www.jacionline.org). In addition, proportions of the ATAC-seq peak regions that represent various genome annotations were not changed between the detergent-exposed and unexposed groups (see Fig E3, A and B).

We further identified promoter regions that were differentially accessible in response to detergent exposure. However, very few regions showed significant differences in accessibility between detergent-exposed and unexposed samples (see Fig E5 in this article's Online Repository at www.jacionline.org). Moreover, there is no common promoter region that was differentially accessible when responding to detergent shared by the control, asthma, and COPD groups, suggesting a random finding (Fig 5, C).

The detailed information of differentially accessible promoter regions in response to detergent A are presented in Table E1 in this article's Online Repository at www.jacionline.org. Supporting these data, multidimensional scaling analysis of chromatin accessibility signals at promoter regions also showed no clear separation between detergent-exposed and unexposed samples (see Fig E6 in this article's Online Repository at www.jacionline.org). We further checked the chromatin accessibility of ZO-1 and occludin, which showed irregular and patchy immunostaining in Fig 1, D. The chromatin accessibility state of ZO-1 and occludin identified by using ATAC-seq peaks did not show any difference between detergent-exposed and unexposed samples in the control, asthma, and COPD groups (Fig 5, D and E). Taken together, these results indicated that the effect of detergent on chromatin accessibility is negligible.

Similarly, no difference was found in global DNA methylation levels, as measured by long interspersed nucleotide element 1 repeats, after 24 hours of detergent exposure (Fig 5, F). The DNA methylation array showed that there were 9951 and 7905 differentially methylated probes (DMPs) responding to detergent exposure in the control and asthma groups, respectively. However, only 0.8% of these DMPs were shared by the control and asthma groups, which indicated a nontargeted effect of the experiment on DNA methylation (Fig 5, G). Furthermore, based on these DMPs,

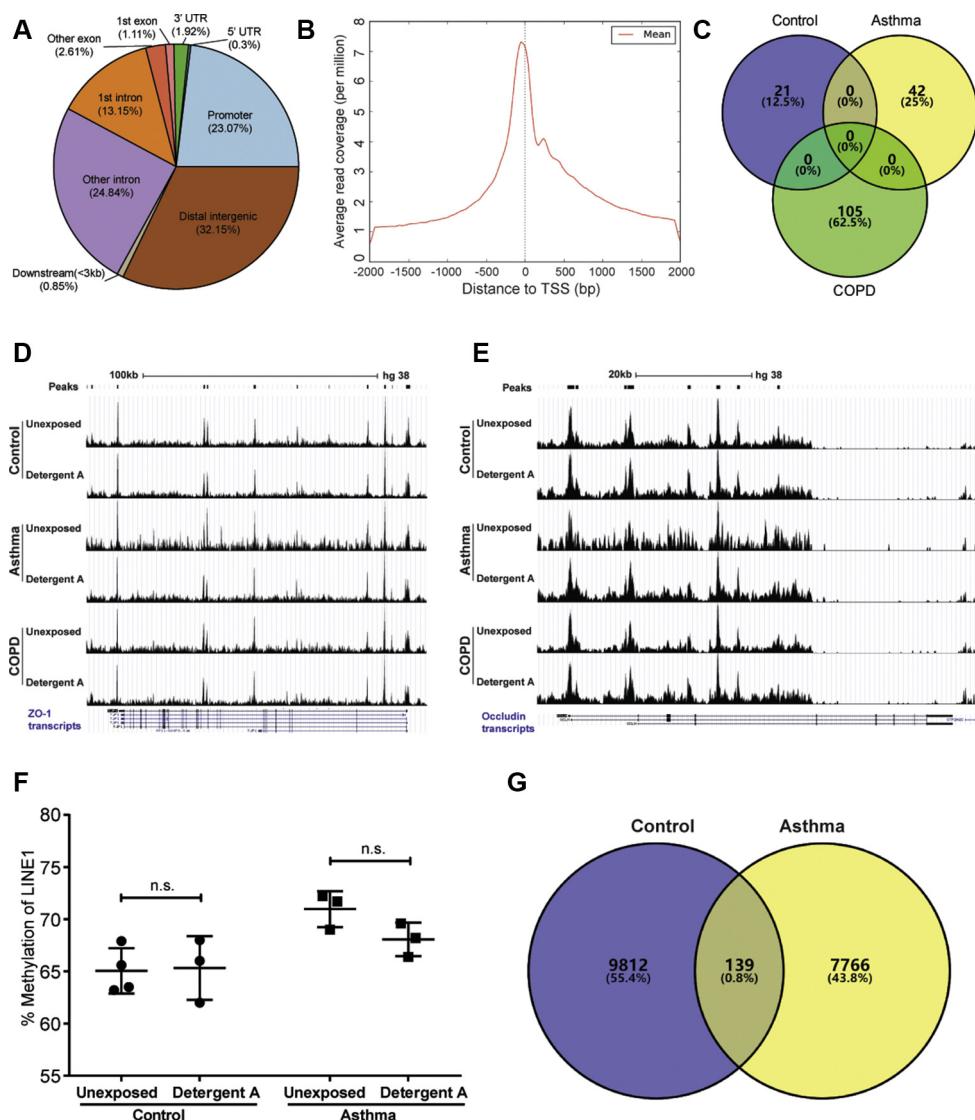


FIG 5. Minor effects of laundry detergent on chromatin accessibility and DNA methylation. **A**, Pie chart showing distribution of ATAC-seq peak regions that represent various genome annotations in HBEs from healthy control subjects. **B**, Distribution of accessible regions within 2 kb of the nearest transcriptional start sites (*TSS*) in HBEs from healthy control subjects. **C**, Venn diagrams illustrating the intersection of differentially accessible regions identified by using ATAC-seq between detergent-exposed and unexposed group in HBEs from healthy control subjects (*n* = 5), asthmatic patients (*n* = 6), and patients with COPD (*n* = 5). Detergent A was used at 1:50,000 dilutions for 24 hours of exposure. **D** and **E**, Sequencing tracks for the ZO-1 and occludin locus showing ATAC-seq peaks in different conditions. **F**, DNA methylation levels of long interspersed nucleotide element 1 (*LINE1*) in HBEs from healthy control subjects and asthmatic patients after 24 hours of exposure to 1:50,000 diluted laundry detergent (*n* = 3 per group). *n.s.*, Not significant. **G**, Venn diagrams depicting intersection of DMPs detected by using the DNA Methylation EPIC array in response to laundry detergent exposure. Numbers of DMPs are marked in the corresponding areas.

no differentially methylated regions or genes responding to detergent exposure were identified, confirming the results of ATAC-seq.

RNA-seq transcriptome response of HBECs to detergents

After 24 hours of exposure to detergent A diluted at 1:50,000, RNA-seq was performed on ALI-cultured HBECs from healthy

control subjects, asthmatic patients, and patients with COPD. DEGs between the unexposed and detergent-exposed groups were identified based on $\log_2$ fold change of greater than 0.5 or less than -0.5 plus a significant P value of less than .01. There are 362, 273, and 417 DEGs responding to detergent exposure in the control, asthma, and COPD groups, respectively (Fig 6, A-D). The detailed information of DEGs in response to detergent A are presented in Table E2 in this article's Online Repository at www.jacionline.org. Eighty-one common DEGs, including 68

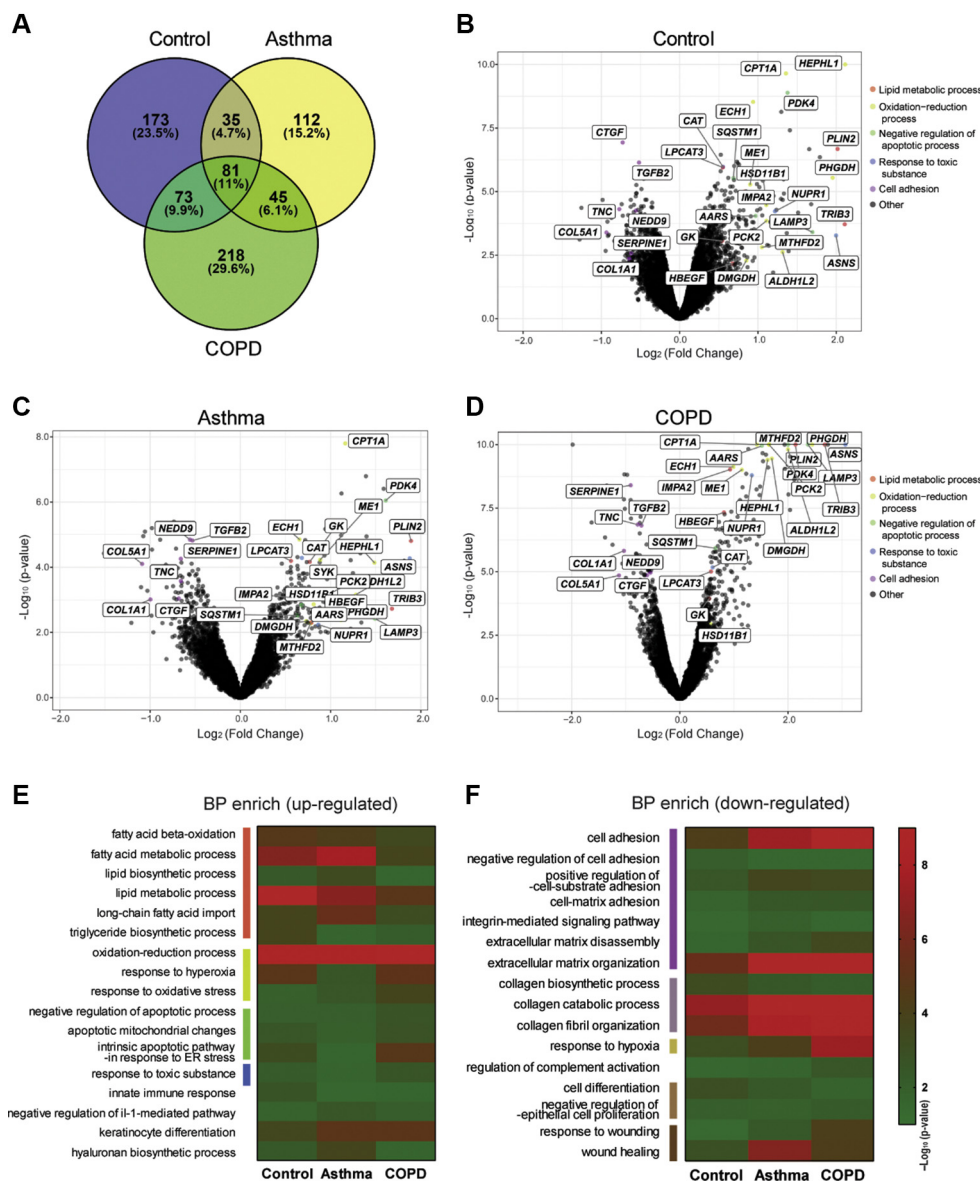


FIG 6. DEGs in response to laundry detergent exposure in HBECS. **A**, Venn diagrams illustrating intersection of DEGs detected by using RNA-seq in response to detergent A at 1:50,000 dilutions in HBECS from healthy control subjects (n = 5), asthmatic patients (n = 6), and patients with COPD (n = 5) after 24 hours of exposure. Numbers of DEGs are marked in the corresponding areas. **B-D**, Volcano plots showing DEGs in response to laundry detergent A exposure in HBECS from healthy control subjects, asthmatic patients, and patients with COPD. DEGs of significantly enriched GO terms were labeled with different colors. **E** and **F**, Significantly common enriched BP of GO terms shared by upregulated DEGs or downregulated DEGs in the control, asthma, and COPD groups. Color coding of heat maps represents the $-\log_{10} P$ value of GO enrichment. Similar BP terms are highlighted by color bars.

upregulated and 13 downregulated DEGs, were shared by the control, asthma, and COPD groups (Fig 6, A, and see Table E3 in this article's Online Repository at www.jacionline.org).

DEGs in the control, asthma, and COPD groups were analyzed for GO term enrichment, respectively, to identify the biological pathways affected by laundry detergents. The biological process (BP) of GO terms enriched by upregulated or downregulated DEGs in the healthy control, asthma, and COPD groups are shown in Fig E7 in this article's Online Repository at www.jacionline.org. The common BP terms shared by 3 groups are shown in Fig 6, E and F. Common BP terms significantly enriched

by upregulated DEGs were mainly associated with lipid metabolism, oxidative stress, and regulation of apoptosis processes, whereas downregulated DEGs were mainly enriched in cell adhesion, extracellular matrix, and collagen-related and wound-healing processes. Similar results were observed in GO term enrichment, with 81 common DEGs shared by the control, asthma, and COPD groups (see Fig E8 in this article's Online Repository at www.jacionline.org).

We further checked the expression of cytokines described as the epithelially derived alarmins IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which trigger type 2 immunity in

response to laundry detergent exposure. The expression level of IL-25 was not detected by means of RNA-seq. IL-33 levels were significantly upregulated by laundry detergent in the control, asthma, and COPD groups. RNA-seq data also showed that TSLP levels were increased in response to laundry detergent in HBECs from healthy control subjects but not in HBECs from asthmatic patients and patients with COPD (see Fig E9 in this article's Online Repository at www.jacionline.org).

mRNA expression of major TJ genes were not affected by laundry detergent

TJs form a continuous seal between the neighboring epithelial cells and are crucial for their barrier function. Therefore we further analyzed the expression of TJ genes, including occludin, claudin family genes, junctional adhesion molecules, and TJ protein family genes. The RNA-seq data showed that the major TJ genes expressed in HBECs were not substantially affected by detergent exposure (see Fig E10 in this article's Online Repository at www.jacionline.org). Consistent with the ATAC-seq result, mRNA expression of ZO-1 and occludin identified by using RNA-seq was not changed in response to detergent exposure (see Fig E11 in this article's Online Repository at www.jacionline.org). These results demonstrate that laundry detergent applied to HBECs for 24 hours directly disrupts TJ proteins and their barrier integrity in HBECs without affecting the expression of TJ genes.

DISCUSSION

Our data demonstrated that both laundry detergents and detergent residue after rinsing showed high cytotoxicity and directly impaired barrier integrity of bronchial epithelial cells. Considering that a defective barrier can increase the risk of tissue penetration of allergens, microbes, and their enzymes and toxins and other environmental pollutants, as well as the succeeding inflammatory reaction, the effect of broadly used detergents on airway epithelium could be one important reason of increased prevalence of asthma in the past few decades.

The bronchial mucosal epithelial barrier is predominantly formed by TJs located at the most apical part of the intracellular junctional complexes between the neighboring epithelial cells.^{31,32} We performed immunofluorescence staining of the TJ proteins occludin and ZO-1 on ALI-cultured HBECs after detergent or rinse residue exposure. Significant disruptions of TJ integrity were found with irregular and patchy staining of occludin and ZO-1. However, there is no obvious change in the mRNA, DNA methylation, and chromatin accessibility in TJs and TJ-related genes in response to detergent exposure. Because of the ability of detergents that generally break lipid-lipid interactions and lipid-protein interactions, which allows membrane proteins to be solubilized and bleached and surface tension and viscosity to be changed,³³ our data suggested that the TJ integrity disruption induced by detergent was instead a direct effect on the barrier molecules. Consistent with this, lipid metabolism-related genes were significantly upregulated by laundry detergent, which could be an effort from epithelial cells to compensate the damage of cell membrane lipids. Interestingly, cell adhesion and collagen synthesis-related genes, such as cadherins and tenascin C, were generally downregulated by detergent, which can also contribute to the disruptive effect of detergents on barrier integrity.^{34,35}

Interaction between the airway epithelium and the inhaled environment is crucial to both the origins and progression of asthma and other types of respiratory inflammation. Evidence is accumulating that airway epithelium-derived cytokines described as epithelium-derived alarmins, IL-25, IL-33, and TSLP, regulate the immune response to inhaled noxious agents and allergens.³⁶ The significant increase in IL-33 levels in ALI cultures of epithelium of control subjects, asthmatic patients, and patients with COPD and TSLP levels in control epithelium suggest a possibility for the induction of type 2 immunity by laundry detergents, which is open to future research.

Laundry detergents showed a high toxic effect to HBECs. Concentrations of greater than 1:40,000 dilution of detergent A led to a clear leakage of cytoplasmic LDH, demonstrating cell death or plasma membrane damage. Even with exposure to a concentration without apparently affecting LDH leakage and cell morphology, RNA-seq results also showed that genes associated with the apoptotic process and response to toxic substances were significantly upregulated. Recent studies demonstrated that laundry detergent pods are more toxic than other types of detergents.^{37,38} Therefore the safety of laundry detergents needs further evaluation. Exposure to toxic doses of detergents to any living organism should be strictly controlled, such as floor and carpets in schools and milk cans, a long list that can be extended to many environmental exposures.

The ingredients of commercial laundry detergents are very complex, including surfactants, builders, bleaches, fragrances, and enzymes. Notably, a number of laundry detergent ingredients have been proved to be irritants or sensitizers. Surfactants and bleach are highly irritating to the skin and lungs. Fumes from bleach have an irritant effect on the airways and can increase bronchial hyperresponsiveness.^{39,40} Enzymes used in laundry detergent are reported as potential respiratory sensitizers.^{12,41} Fragrance can also cause exacerbations of symptoms and airway obstruction in asthmatic patients.⁴²

Because retention of allergens or compounds in clothing is associated with allergic disease,⁴³ we checked the detergent residue in clothing after washing. Our result indicated a large amount of detergent residue in clothing demonstrated by concentration of residual SDBS and protease. Despite further dilution at 1:10 or 1:5 after approximately 1:2500 dilution of rinsing, rinse residue showed clearly toxic and barrier-disruptive effects. Considering the high concentration and large amount of detergent residue in clothing and the close contact with human organs, the irritative and sensitized ingredients of laundry detergents can be easily inhaled into the airways and reach the lung from newly washed clothing. Moreover, evidence showed that frequent use of common household cleaning sprays were also an important risk factor for adult asthma among both household owners and professional cleaning personnel,^{9,44} which also supports evidence that detergents can be inhaled in a household.

Accordingly, we measured manual aerosolization of SDBS with a manual dispenser. Concentrations of SDBS with different carbons in commercial laundry detergent was measured by UPLC. Spray of a 1:25,000 dilution still contained detectable amounts of carbon 10, 11, 12, and 13 SDBS (see Fig E12 in this article's Online Repository at www.jacionline.org). It must be noted that contents of household detergents, carpet cleaners, dishwashers, and laundry detergents are quite similar, indicating extensive exposure to detergents. Therefore the problem of

long-term high exposure to detergent in daily life should receive attention for public health.

Epigenetic mechanisms are thought to play a role in different complex diseases of the lungs, such as asthma and COPD, which are strongly influenced by environmental factors, such as cigarette smoke.⁴⁵⁻⁴⁷ Although we showed a negligible effect of laundry detergents on chromatin accessibility and DNA methylation in HBECS, these data helped us reach the conclusion that laundry detergents directly attack barrier integrity, without affecting the epigenome in short-term exposure. However, we cannot rule out the possibility that a longer time period and more chronic daily exposure to detergent could influence DNA methylation. In line with this concept, one recent study demonstrated that exposure of HBECS to diesel exhaust had minimal effects on DNA methylation at 48 hours; however, when the same lung was exposed to diesel exhaust but separated by 4 weeks, significant changes in DNA methylation were observed.⁴⁸ Recently, the investigation of dynamic DNA methylation changes at single-cell resolution showed that DNA methylation undergoes gradual changes over the time course of 7 days during development.⁴⁹ Therefore detection of the epigenome during the pathogenesis of diseases at a single time point might be insufficient. This study reveals data necessitating long-term exposure and real-life studies.

In summary, the present study demonstrated that laundry detergents have significantly toxic and directly disruptive effects on the TJ barrier integrity of HBECS without affecting chromatin accessibility, DNA methylation, and mRNA expression of TJ genes, which provided a possible mechanism for laundry detergents causing asthma and allergic disease. Furthermore, the present study showed high amounts of detergent residue in clothing after laundry and a TJ-damaging effect of detergent residue. Further studies are needed to test long-time high exposure to detergents in daily life and the accumulated effect on epithelial barriers, such as the nose, lung, skin, and gut.

Key messages

- Laundry detergents and rinse residue disrupted the TJ barrier integrity of HBECS from healthy control subjects, asthmatic patients, and patients with COPD.
- Single 24-hour laundry detergent exposure at 1:50,000 dilution showed negligible effects on DNA methylation and chromatin accessibility.
- Lipid metabolism, apoptosis progress, and epithelially derived alarmin-related genes were upregulated, whereas cell adhesion-related genes were downregulated by 1:50,000 times diluted laundry detergent in RNA-seq.

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ONLINE REPOSITORY

Laundry detergents and detergent residue after rinse directly disrupt tight junction barrier integrity in human bronchial epithelial cells

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METHODS

Measurement of residual detergent enzymes in clothing

To investigate the levels of residual detergent enzymes in clothing, clothing with three different fabrics, cotton, polyester and wool, were collected. Normal laundry was performed with a recommended amount of detergent. Each set of samples were washed and dried with regular clothes to reflect real-world situations. The commercially available enzyme assay, Subtilisin A from *Bacillus Licheniformis* (Megazyme International Ireland, Bray, County Wicklow, Ireland), were used to measure levels of protease (a typical detergent enzyme) in the original liquid detergent and weighed clothing samples. The levels of protease per unit mass of sample were calculated as suggested by the manufacturer's instructions.

Endotoxin assay

Endotoxin assay was performed to exclude possible contamination of endotoxin in detergent reagent used for cell exposure. Endotoxin content in different dilutions of laundry detergent (1:2500, 1:25,000 and 1:50,000) and rinse residue liquid collected from newly washed clothes (1:5 and 1:10) were determined using ToxinSensor Chromogenic Limulus Amebocyte Lysate Endotoxin Assay Kit (GenScript, Piscataway, USA) according to the manufacturer's instruction. The detection range of used kit was 0.01 - 1.00 EU/ml. Endotoxin levels were analyzed with automated light absorbance reader (Mithras LB940, Berthold Technologies). Results were calculated according to the standard curve by MicroWin Software.

Aerosolization assay of laundry detergent

Laundry detergent A was diluted by distilled water at 1:2,500, 1:25,000 and 1:50,000 (vol/vol). Aerosolization experiment of detergent A dilutions were performed by using a manual dispenser. The detergent suspensions were sprayed, and the particles were collected and analyzed by using ultra performance liquid chromatography (UPLC) (ACQUITY UPLC H-Class Bio System, Waters Corporation, USA). Then the concentration of sodium dodecyl benzene sulphonate (SDBS) with different carbons (C10, C11, C12 and C13) were calculated. Serial dilutions of SDBS chemical was used to make the standard curve.

SUPPLEMENTAL FIGURES

FIG E1. Residual detergent enzymes in clothing. The level of protease (Subtilisin A from *Bacillus Licheniformis*, a typical detergent enzyme) was measured in the original liquid detergent and weighed clothes with three different fabrics (cotton, polyester and wool) after a normal laundry. Bar graph shows the levels of protease per unit mass of sample.

FIG E2. Endotoxin levels in different dilutions of laundry detergent and rinse residue. Limulus Amebocyte Lysate Test was performed in different dilutions of laundry detergent (1:2500, 1:25,000 and 1:50,000) and rinse residue liquid collected from newly washed clothes (1:5 and 1:10) (n=4 per group in duplicates).

FIG E3. Distribution of chromatin accessible regions identified using ATAC-seq. **A** and **B**, Pie chart showing the proportions of the ATAC-seq peak regions that represent the various genome annotations in unexposed (A) and detergent A exposed (B) HBECs from healthy control subject. Downstream refers to center of ATAC-seq peaks located less than 3 kb from the transcriptional termination sites. Distal intergenic regions refer to ATAC-seq peaks located more than 3 kb away from the transcriptional start sites (TSS) and more than 1 kb from the transcriptional termination sites. **C**, Heat map showing the distribution of all ATAC-seq peaks within 2 kb of the nearest TSS in HBECs from healthy control subject.

FIG E4. Correlation of ATAC-seq signals between all samples. Spearman's correlation coefficient was calculated between all samples from control, asthma and COPD groups based on read coverage. There is no clear separation between detergent exposed and unexposed samples.

FIG E5. Differentially accessible promoter regions identified by ATAC-seq. **A-C**,

Volcano plot illustrating differentially accessible regions at promoter identified by ATAC-seq between detergent exposed and unexposed group in HBECs from healthy control subjects (n=5), asthma (n=6) and COPD patients (n=5). Red color represents the significant promoter region with p-value < 0.01 plus $\log_2(\text{Fold Change}) > 0.5$ or < -0.5.

FIG E6. Multidimensional scaling analysis of chromatin accessibility signals at promoter regions. Multidimensional scaling analysis was performed with ATAC-seq signals at promoter regions in all samples from control, asthma and COPD groups. There is no clear separation between detergent exposed and unexposed samples.

FIG E7. Significantly enriched biological process terms by DEGs in response to laundry detergent A in HBECs. **A-F,** Top 20 significant biological process (BP) of GO terms enriched by up-regulated or down-regulated DEGs in HBECs from healthy control subjects (A and B), asthma (C and D) and COPD (E and F) patients.

FIG E8. Significantly enriched biological process terms by common DEGs responding to laundry detergent A. Top 10 BP terms significantly enriched by 81 common DEGs shared by control, asthma and COPD group in response to detergent A exposure. BP terms with p-value < 0.05 were considered statistically significant and plotted as $-\log_{10}(\text{p-value})$.

FIG E9. Expression levels of IL-33 and TSLP in response to laundry detergent. Epithelial-derived IL-33 and TSLP expression in RNA-seq in HBECs from healthy control subjects (n=5), asthma (n=5) and COPD patients (n=5) in the presence of 1:50,000 diluted commercial detergent and unstimulated conditions. FPKM, Fragments Per Kilobase Million.

FIG E10. Fold changes of mRNA expression of Tight Junction genes regulated by detergent A. Heat map plotted as $\log_2(\text{Fold Change})$ of Tight Junction genes in

response to detergent A exposure measured by RNA-seq. * p-value <0.05.

FIG E11. Expression level of ZO-1 and Occludin in different conditions. **A** and **B**, ZO-1 and Occludin expression identified by RNA-seq in HBECs from healthy control subjects (n=5), asthma (n=6) and COPD patients (n=5). FPKM, Fragments Per Kilobase Million.

FIG E12. Concentrations of sodium dodecyl benzene sulphate (SDBS) in aerosolized laundry detergent. Different diluted laundry detergent A were aerosolized using a manual dispenser and analyzed by UPLC. The concentration of SDBS with different carbons (C10, C11, C12 and C13) were calculated. Spray of a 1:25,000 dilution still contained detectable amounts of C10, C11 and C12 SDBS.

ONLINE REPOSITORY

Laundry detergents and detergent residue after rinse directly disrupt tight junction barrier integrity in human bronchial epithelial cells

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SUPPLEMENTAL TABLES

Table E1. Differentially accessible promoter regions identified by ATAC-seq in response to laundry detergent.

Control			Asthma			COPD		
Gene	Log ₂ (fold change)	p-Value	Gene	Log ₂ (fold change)	p-Value	Gene	Log ₂ (fold change)	p-Value
LILRB5	0.600	0.00046	SLA2	-0.674	0.00059	USP49	-0.675	0.00008
MCOLN3	-0.587	0.00047	ZNF268	0.587	0.00064	LTB4R	0.738	0.00015
AL355377.2	-0.594	0.00050	GRXCR2	0.600	0.00073	NT5C	-0.620	0.00020
FOXC2	0.572	0.00055	AP006621.5	-0.560	0.00135	CIDEB	0.707	0.00022
CDH23	-0.588	0.00076	PCYT2	-0.558	0.00137	C6orf106	-0.536	0.00025
ISYNA1	-0.544	0.00085	SLC16A12	-0.635	0.00139	PNPLA4	0.632	0.00026
NOL12	-0.538	0.00133	TNPO2	-0.562	0.00163	LCE3A	0.840	0.00032
CENPT	-0.520	0.00135	UBE2R2	-0.545	0.00169	NATD1	-0.758	0.00039
AC245427.5	0.562	0.00141	KIF3C	-0.526	0.00185	FAM210A	0.511	0.00045
FBRSL1	-0.533	0.00150	TIGD7	-0.527	0.00192	DHCR7	-0.527	0.00048
KRTAP9-6	-0.558	0.00167	CRYBG2	-0.594	0.00200	RNMT	0.504	0.00053
CELA2B	-0.531	0.00233	S100A16	-0.538	0.00202	AC135584.1	0.727	0.00053
GRIN1	-0.509	0.00239	SMKR1	0.528	0.00203	IGHD1-7	0.825	0.00054
HHATL	0.527	0.00240	PLEKHG6	0.524	0.00210	MT-CO1	0.551	0.00068
CFHR5	0.518	0.00302	CYTL1	-0.641	0.00217	COMMD5	0.660	0.00074
BTNL2	-0.510	0.00321	KLHL8	0.599	0.00223	MARVELD3	0.602	0.00083
LYZ	0.503	0.00321	AC087164.2	-0.598	0.00254	NRK	0.757	0.00090
LCN10	-0.506	0.00325	AC009090.4	0.612	0.00291	RAVER2	-0.709	0.00091
TMPRSS2	0.535	0.00326	MYL9	-0.505	0.00298	HEPN1	0.770	0.00093
AC244034.3	-0.526	0.00380	C14orf93	0.567	0.00313	USF1	-0.562	0.00095
AADACL2	0.502	0.00517	KRTAP19-3	0.528	0.00318	BTBD19	0.647	0.00098
			HPSE	0.501	0.00326	FOXR1	0.767	0.00099
			CENPL	0.516	0.00342	SEC14L6	0.745	0.00105
			AL158070.2	0.530	0.00344	SLC35G1	-0.577	0.00110
			HAND2	0.600	0.00347	MT-CO2	0.528	0.00115
			TLR10	-0.597	0.00350	AC018445.4	0.768	0.00131
			PDLIM3	0.514	0.00405	UNG	0.672	0.00132
			AC011530.1	-0.514	0.00407	TRMT10A	-0.532	0.00132
			TERB1	-0.542	0.00434	AL353588.1	0.689	0.00140
			ADAMTS4	0.560	0.00437	PRPH	0.620	0.00146
			ZNF197	-0.502	0.00452	HHEX	-0.520	0.00152
			CSDC2	-0.507	0.00465	AL929554.1	0.708	0.00152
			AAED1	-0.509	0.00468	MT-ND2	0.507	0.00155
			TRAV6	-0.503	0.00510	DPYS	0.710	0.00163
			ACKR1	0.566	0.00602	MT-ND1	0.545	0.00163

MTA1	0.510	0.00634	ORAI1	-0.507	0.00176
NPTX2	-0.509	0.00648	RNASE8	0.718	0.00176
ABHD2	-0.533	0.00657	MT-CO3	0.525	0.00177
AC233723.2	-0.543	0.00703	HIST1H2BF	-0.519	0.00189
TTC24	-0.554	0.00731	ATAD3B	-0.522	0.00191
TANK	0.501	0.00815	ELOB	-0.525	0.00193
SIM1	0.506	0.00862	MT-ND5	0.528	0.00198
			AL162377.2	0.741	0.00203
			MT-ATP8	0.514	0.00205
			MT-CYB	0.526	0.00206
			CLTCL1	-0.596	0.00210
			MT-ND6	0.527	0.00210
			MT-ATP6	0.507	0.00219
			ENOX1	0.599	0.00233
			CYTIP	-0.625	0.00233
			PMP22	0.713	0.00241
			MT-ND3	0.516	0.00245
			BFSP2	0.722	0.00251
			C10orf107	0.595	0.00265
			MRPS27	-0.530	0.00266
			MIDN	0.508	0.00284
			MT-ND4	0.519	0.00285
			REEP2	-0.571	0.00288
			PKIG	-0.614	0.00294
			SEPT6	-0.589	0.00294
			MT-ND4L	0.512	0.00328
			CAMK2N2	-0.575	0.00335
			TIAF1	-0.533	0.00354
			AEBP2	0.671	0.00359
			WBSCR28	0.630	0.00371
			STON1	0.692	0.00378
			LRRC30	0.667	0.00408
			SEC31B	-0.643	0.00410
			POU4F1	0.545	0.00422
			AC007683.2	-0.596	0.00451
			SLC26A4	0.593	0.00481
			PIGQ	0.529	0.00483
			NHLRC4	0.529	0.00483
			PDE2A	0.599	0.00509
			MATK	-0.628	0.00528
			LGI2	0.626	0.00538
			ADGRL4	0.657	0.00557
			RTEL1	-0.571	0.00570
			-TNFRSF6B		

	PLD4	0.608	0.00592
	LRFN2	-0.526	0.00593
	PHYHIPL	0.592	0.00602
	CTDSPL	-0.503	0.00638
	MEFV	0.607	0.00650
	PLCXD3	0.581	0.00651
	CLEC4C	0.606	0.00691
	TRBJ2-2	0.579	0.00711
	PRKAG3	0.576	0.00741
	EPHA4	0.554	0.00741
	NKG7	0.615	0.00754
	RSPH9	-0.522	0.00755
	TRAJ46	0.658	0.00763
	AL355987.1	0.556	0.00834
	UGT3A2	0.556	0.00838
	ETNPPL	0.540	0.00856
	CYP21A2	-0.535	0.00921
	AC034102.2	0.563	0.00925
	LRFN3	-0.577	0.00941
	ABL1	-0.558	0.00944
	AC004836.1	0.604	0.00950
	SLC9A7	-0.556	0.00955
	SULT1E1	-0.532	0.00963
	C5AR2	-0.517	0.00980
	ZNF629	0.513	0.00991
	PROZ	0.593	0.00992
	TRBJ2-3	0.604	0.00994

Table E2. Differentially expressed genes identified by RNA sequencing in response to laundry detergent.

Control			Asthma			COPD		
Gene	Log ₂ (fold change)	p-Value	Gene	Log ₂ (fold change)	p-Value	Gene	Log ₂ (fold change)	p-Value
HEPHL1	2.113	0.00000	CPT1A	1.163	0.00000	ASNS	3.061	0.00000
CPT1A	1.357	0.00000	CRCT1	1.392	0.00000	TRIB3	2.681	0.00000
PDK4	1.378	0.00000	CRNN	1.890	0.00000	PLIN2	2.139	0.00000
ECH1	0.938	0.00000	TM6SF1	1.588	0.00000	PHGDH	2.448	0.00000
MMP1	1.298	0.00000	CSF2	1.121	0.00000	PCK2	1.649	0.00000
C6orf223	0.787	0.00000	PDK4	1.611	0.00000	CPT1A	1.427	0.00000
SPRR1A	1.407	0.00000	TGFBI	-0.736	0.00000	ADM2	1.938	0.00000
CTGF	-0.727	0.00000	ADAMTS9	0.970	0.00001	PSAT1	2.738	0.00000

PLIN2	2.016	0.00000	NDUFA4L2	-0.980	0.00001	MTHFD2	1.650	0.00000
TM6SF1	1.654	0.00000	ATP6V0D2	0.942	0.00001	LAMP3	2.369	0.00000
AC008982.1	0.525	0.00000	LOXL2	-0.903	0.00001	COL1A2	-1.983	0.00000
SLCO4A1	1.042	0.00000	FAT4	-1.057	0.00001	PDK4	2.002	0.00000
CAPN14	1.791	0.00000	CCL22	1.117	0.00001	AARS	1.516	0.00000
FADS1	0.732	0.00000	SPOCK2	-1.075	0.00001	SLC6A9	1.266	0.00000
TGFB2	-0.519	0.00000	SULT1C2	1.398	0.00001	ALDH1L2	2.000	0.00000
CX3CL1	0.743	0.00000	ECH1	0.655	0.00001	SLC7A11	1.530	0.00000
IL1RN	0.759	0.00000	NEDD9	-0.559	0.00001	MOCOS	1.592	0.00000
HAS3	0.696	0.00000	TENM2	-0.889	0.00001	DMGDH	1.701	0.00000
CAT	0.553	0.00000	SLC29A1	0.719	0.00001	HEPHL1	1.623	0.00000
LPCAT3	0.550	0.00000	TGFB2	-0.531	0.00002	BEST1	1.539	0.00000
SPRR3	1.359	0.00000	PLIN2	1.892	0.00002	ECH1	0.991	0.00000
SPRR1B	0.821	0.00000	THSD7A	1.219	0.00002	DDIT3	1.633	0.00000
SCD	0.523	0.00000	HPGD	0.803	0.00002	TM6SF1	1.945	0.00000
CSTB	0.826	0.00000	SPARC	-0.713	0.00003	IMPA2	0.929	0.00000
SYK	0.615	0.00000	C4orf26	-0.979	0.00003	ME1	1.148	0.00000
PHGDH	1.954	0.00000	PRSS3	0.748	0.00003	CLGN	1.227	0.00000
SQSTM1	0.687	0.00000	IL36RN	1.118	0.00003	COL4A1	-1.025	0.00000
IL36RN	1.327	0.00000	SPRR3	1.331	0.00004	COL5A2	-0.974	0.00000
LY6D	0.694	0.00000	COL1A2	-1.510	0.00004	NUPR1	1.332	0.00000
SMOC1	1.063	0.00000	CA9	-1.068	0.00004	CHAC1	2.117	0.00000
LCP1	0.988	0.00001	IL1R2	0.932	0.00004	SERPINE1	-0.909	0.00000
ME1	0.900	0.00001	CEL	-0.559	0.00004	SPARC	-1.352	0.00000
PIP	-0.553	0.00001	ETFDH	0.633	0.00005	IFRD1	1.484	0.00000
CEACAM1	0.682	0.00001	CAT	0.682	0.00005	UPP1	1.159	0.00000
ADAM8	0.676	0.00001	ASNS	1.877	0.00005	WARS	1.418	0.00000
MFSD4A	0.646	0.00001	SERPINE1	-0.659	0.00005	CRCT1	2.009	0.00000
PRSS3	1.019	0.00001	ME1	0.885	0.00006	CSF2	1.476	0.00000
KCNG1	1.523	0.00001	CAPN14	1.223	0.00006	MARS	1.227	0.00000
FADS2	0.538	0.00001	PSAT1	1.816	0.00006	FN1	-1.035	0.00000
ABCC3	0.717	0.00001	LPCAT3	0.561	0.00006	COL4A2	-0.909	0.00000
ETFDH	0.584	0.00001	GK	0.769	0.00007	GARS	1.114	0.00000
MARS	0.848	0.00001	SLC5A1	0.736	0.00007	STC2	1.376	0.00000
IMPA2	0.828	0.00001	RAB3B	0.880	0.00007	VCAN	-1.017	0.00000
GRHL3	0.649	0.00001	MT1E	-0.652	0.00007	KLHDC7B	2.037	0.00000
KLHDC7B	1.461	0.00001	HEPHL1	1.486	0.00007	XPOT	0.952	0.00000
VCAN	-0.552	0.00001	THBS1	-0.540	0.00008	HBEGF	0.816	0.00000
CDHR3	-0.553	0.00001	COL5A1	-1.088	0.00008	MT-ATP8	0.826	0.00000
HDAC9	0.571	0.00001	NXPH4	-0.825	0.00008	ENC1	-0.726	0.00000
CRCT1	1.730	0.00002	PKIB	0.632	0.00009	IARS	1.069	0.00000
CDH6	-0.561	0.00002	SERPINB2	0.560	0.00010	TENM2	-0.903	0.00000
CAPN12	0.854	0.00002	SPNS2	0.805	0.00010	FAM129A	1.366	0.00000
SLCO4C1	0.673	0.00002	IFRD1	0.866	0.00010	HKDC1	0.943	0.00000

PCSK9	0.623	0.00002	COL4A1	-0.693	0.00011	KDR	-1.101	0.00000
BEST1	1.294	0.00002	SYT8	-0.814	0.00011	YARS	0.724	0.00000
WARS	0.979	0.00002	GRAMD3	0.638	0.00011	MMP12	-1.632	0.00000
MB	-0.618	0.00002	SERPINB10	0.555	0.00013	COL7A1	-0.740	0.00000
APOBEC3A	1.317	0.00002	NMB	-0.530	0.00013	C6orf223	0.922	0.00000
HSD11B2	-0.984	0.00002	CHRNA1	0.523	0.00014	KIF21B	1.391	0.00000
UPK3B	0.587	0.00002	ACAA2	0.562	0.00015	TNC	-0.780	0.00000
SHMT2	0.624	0.00002	LCP1	1.248	0.00015	S100P	0.858	0.00000
RCAN1	0.798	0.00002	SLC25A20	0.705	0.00015	TGFB2	-0.711	0.00000
MGAM	-0.740	0.00003	SLC28A3	0.692	0.00016	RNASE7	1.025	0.00000
CHAC1	1.679	0.00003	HAS2	0.740	0.00016	SPRR1A	1.184	0.00000
TFCP2L1	-0.583	0.00003	HCFC1R1	-0.568	0.00016	ANXA10	1.046	0.00000
HSD11B1	1.107	0.00003	OLFML2A	-0.562	0.00017	MFSD4A	0.792	0.00000
MMP10	0.569	0.00004	SERPINB7	0.650	0.00018	SLC1A5	0.669	0.00000
SLC2A6	0.536	0.00004	VSTM2L	-0.664	0.00018	SHMT2	0.735	0.00000
AQP3	0.513	0.00004	C21orf58	-0.797	0.00018	MT-ND1	0.802	0.00000
TRNP1	-0.624	0.00004	ANXA10	0.966	0.00018	SES2	0.868	0.00000
VNN3	1.029	0.00005	TNFRSF19	-0.760	0.00019	MB	-0.758	0.00000
GRAMD3	0.569	0.00005	MT1X	-0.702	0.00020	GTPBP2	0.947	0.00000
TNC	-0.770	0.00005	PNCK	-0.643	0.00020	SLC1A4	1.139	0.00000
ALDH3B1	-0.531	0.00005	ADM2	1.424	0.00020	ULBP1	1.464	0.00000
DUSP5	1.236	0.00005	TUBE1	0.654	0.00022	COL3A1	-1.383	0.00000
HAS2	0.815	0.00005	SYK	0.712	0.00022	TUBE1	1.041	0.00000
C8orf4	0.767	0.00006	C8orf4	0.562	0.00022	INHBE	1.291	0.00000
NEDD9	-0.559	0.00006	S100P	0.962	0.00023	NTNG1	1.222	0.00000
NUPR1	1.218	0.00006	IL36G	0.691	0.00025	CX3CL1	0.746	0.00000
FDPS	0.521	0.00006	CLGN	1.481	0.00026	GDF15	1.112	0.00000
SLC4A4	-0.503	0.00006	TNC	-0.659	0.00026	AIFM2	0.885	0.00000
LRTOMT	-0.586	0.00006	CLIC5	-0.648	0.00030	SPRR1B	0.844	0.00000
ESM1	1.513	0.00007	ALDH3A1	-0.582	0.00030	SLC35F6	0.781	0.00000
MT-ND1	0.624	0.00007	HKDC1	1.146	0.00031	TGFB1	-0.615	0.00000
RTKN2	0.586	0.00008	EGF	0.649	0.00031	PCDH19	-1.245	0.00000
PSAT1	2.103	0.00008	C6orf223	0.568	0.00034	RCAN1	0.902	0.00000
ANXA9	-0.657	0.00008	MB	-0.647	0.00036	MT-ATP6	0.704	0.00000
BEX2	1.055	0.00009	CEACAM1	0.677	0.00037	SQSTM1	0.656	0.00000
RHOF	0.646	0.00009	RRAD	-0.744	0.00038	SLC7A5	0.735	0.00000
LGALS7	0.567	0.00009	LCE3D	0.760	0.00040	COL1A1	-1.035	0.00000
LDLRAD1	-0.726	0.00009	COL8A1	-0.536	0.00040	SARS	0.648	0.00000
CHCHD10	0.591	0.00009	LRRC26	-1.100	0.00041	RBCK1	0.671	0.00000
AARS	0.969	0.00009	GPR162	-0.817	0.00041	FAM102A	0.618	0.00000
N4BP3	0.751	0.00009	GCLC	0.613	0.00041	CEL	-0.810	0.00000
SPNS2	0.909	0.00009	MT-ATP6	0.521	0.00041	CDH11	-0.804	0.00000
AFAP1L1	0.656	0.00010	TRIM31	0.714	0.00043	CXCL2	0.741	0.00000
GPT2	0.683	0.00010	TMEM154	0.501	0.00045	SLFN11	-0.742	0.00000

IL34	0.752	0.00011	RINL	0.561	0.00047	CARS	0.762	0.00000
CBSL	1.226	0.00011	CPT1B	0.654	0.00047	ERN1	0.826	0.00000
CNTNAP2	-0.565	0.00011	GRPEL2	0.987	0.00048	GAB2	0.787	0.00000
HS3ST3B1	-0.871	0.00011	MFSD4A	0.528	0.00049	GCLC	0.565	0.00000
GRPEL2	0.815	0.00012	KISS1R	-1.030	0.00052	SLC7A1	0.607	0.00000
IARS	0.756	0.00012	NQO1	0.755	0.00055	LPO	1.251	0.00000
CSF1	0.629	0.00012	SERPINB4	0.529	0.00056	UPK3B	0.922	0.00000
ARG2	0.768	0.00012	RENBP	0.828	0.00057	LONP1	0.656	0.00000
ATF4	0.659	0.00013	PCK2	1.027	0.00057	LARP6	0.678	0.00000
ACSL1	0.574	0.00013	IFITM10	-0.653	0.00058	GPT2	0.727	0.00000
SLC25A20	0.531	0.00013	MT2A	-0.717	0.00058	EFEMP1	-0.564	0.00000
YARS	0.585	0.00013	CASP5	1.040	0.00059	RNF187	0.583	0.00000
NDRG4	0.847	0.00013	VNN3	0.667	0.00060	PLIN4	1.199	0.00000
HES6	0.597	0.00013	DGAT2	0.608	0.00060	VNN3	1.296	0.00000
GAB2	0.567	0.00013	CNFN	-0.687	0.00061	KANK4	-1.064	0.00000
ADAMTSL3	-0.556	0.00014	ADAMTS6	0.552	0.00063	CAPN12	0.867	0.00001
ECT2L	-0.637	0.00014	ABCC3	0.563	0.00063	CYBRD1	-0.736	0.00001
PCK2	1.109	0.00014	DEGS2	-0.548	0.00065	MMP7	-0.733	0.00001
PHLDA2	0.845	0.00014	CDK5R1	0.518	0.00065	IL1R1	-0.686	0.00001
IL36A	1.164	0.00015	PIP5K1B	0.675	0.00067	CAT	0.606	0.00001
CD4	-0.922	0.00015	LRP1	-0.567	0.00067	HS3ST3B1	-0.745	0.00001
MUC21	0.928	0.00016	GAB2	0.543	0.00068	SPRR3	1.186	0.00001
NTNG1	1.161	0.00016	PPARGC1A	0.617	0.00069	LRRC49	0.824	0.00001
MT-ATP8	0.944	0.00016	ALDH1L2	1.280	0.00069	LTBP1	-0.579	0.00001
CSF3	1.281	0.00017	CLDN10	0.511	0.00070	NPNT	-0.572	0.00001
IL36G	0.851	0.00018	KCNMA1	-0.603	0.00072	MAGED1	-0.532	0.00001
HOPX	0.726	0.00019	RNASE7	0.892	0.00074	MMP2	-0.591	0.00001
METRNL	0.575	0.00019	LOX	-0.591	0.00075	RGS3	0.705	0.00001
TRIB3	2.108	0.00019	KIF21B	0.822	0.00076	ABI3BP	-0.568	0.00001
ELOVL7	0.532	0.00020	TRPV3	0.868	0.00078	ATP13A4	0.743	0.00001
FAT4	-0.677	0.00021	PFKFB3	-0.596	0.00080	MUC21	0.946	0.00001
GARS	0.849	0.00021	SERTAD4	-0.543	0.00081	TNFSF10	-0.557	0.00001
SNTN	-0.678	0.00021	MOCOS	0.884	0.00081	LPCAT3	0.575	0.00001
CIB2	0.560	0.00022	ESM1	0.714	0.00083	PYCR1	0.699	0.00001
MSMB	-0.570	0.00022	IMPA2	0.565	0.00085	IL1R2	0.966	0.00001
ATP6V1B1	-0.575	0.00023	ANLN	-0.733	0.00089	NEDD9	-0.543	0.00001
STARD13	-0.524	0.00023	ACSL3	0.505	0.00090	POSTN	-0.715	0.00001
FTH1	0.536	0.00025	CTGF	-0.682	0.00090	FLT1	-0.857	0.00001
SBSN	0.751	0.00025	COL1A1	-0.997	0.00097	SLC25A20	0.747	0.00001
GDF15	1.548	0.00025	TMEM255A	-0.707	0.00097	CDR2	-0.643	0.00001
SYT8	-0.502	0.00026	GDPD1	0.608	0.00102	COL12A1	-0.664	0.00001
TRPV3	0.951	0.00030	ARHGAP20	-0.588	0.00104	CTGF	-0.606	0.00001
DZIP3	-0.505	0.00032	COL7A1	-0.650	0.00107	COL5A1	-1.126	0.00001
PDGFD	-0.578	0.00032	RCAN1	0.609	0.00113	TSPAN12	-0.653	0.00001

PRKAR2B	-0.675	0.00033	KRT75	0.920	0.00114	CBS	1.057	0.00002
CYP3A5	0.916	0.00034	COL12A1	-0.516	0.00117	PAPPA	-0.998	0.00002
XPOT	0.641	0.00036	MARS	0.556	0.00117	ODC1	0.638	0.00002
PIK3R3	0.503	0.00036	P3H3	-0.522	0.00118	FTH1	0.579	0.00002
GTPBP2	0.709	0.00037	SHC4	0.521	0.00119	GRPEL2	0.569	0.00002
ARMC4	-0.514	0.00038	SPRR1B	0.536	0.00126	ACSL1	0.554	0.00002
ZNF598	0.536	0.00039	INA	-0.568	0.00126	HSD11B2	-0.731	0.00002
LAMP3	1.696	0.00040	C11orf96	0.949	0.00126	LMAN1	-0.502	0.00002
KCNRG	-0.672	0.00040	COL4A2	-0.581	0.00132	KCNG1	1.017	0.00002
COL5A1	-0.935	0.00041	WARS	0.673	0.00134	ARG2	0.723	0.00002
FAM129A	0.713	0.00041	HSD11B1	0.813	0.00137	PSPH	0.783	0.00002
SNTB1	0.579	0.00042	MXRA5	-0.621	0.00140	MTSS1	-0.509	0.00003
ANKRD66	-0.666	0.00042	AARS	0.678	0.00146	PTHLH	-0.620	0.00003
SLC1A4	0.730	0.00042	FAR2	0.692	0.00148	CDH6	-0.593	0.00003
DTHD1	-0.620	0.00042	LRP4	-0.533	0.00154	TMEM154	0.556	0.00003
ADM2	1.368	0.00043	NKX3-1	-0.612	0.00159	SPOCK1	-0.608	0.00003
MT-ATP6	0.685	0.00044	KLK5	0.636	0.00159	CXCR4	-0.956	0.00003
SPRR2A	1.015	0.00046	MRC2	-0.544	0.00161	LOXL2	-0.584	0.00003
LCE3E	1.009	0.00047	PRIMA1	-0.724	0.00165	MT-ND4L	0.542	0.00003
ELOVL4	0.649	0.00047	DLL1	-0.550	0.00169	SLC4A4	-0.731	0.00004
EDN2	-0.502	0.00048	APOBEC3A	0.878	0.00172	VTCN1	-0.556	0.00004
PCDH19	-0.904	0.00050	CTH	0.695	0.00173	BBC3	0.581	0.00004
SFXN2	-0.616	0.00050	CRLF1	-0.535	0.00173	SLC5A1	0.723	0.00004
MVD	0.528	0.00050	KRT34	0.831	0.00184	GABRP	-0.525	0.00004
DNAH6	-0.676	0.00052	PLA2G4F	0.559	0.00185	PTGES2	0.547	0.00004
DNAH12	-0.592	0.00053	TRIB3	1.680	0.00186	TLN2	1.130	0.00004
SLC28A3	0.534	0.00053	RNASE1	0.635	0.00187	CDC42EP2	0.637	0.00004
ASNS	1.997	0.00053	CEACAM7	0.753	0.00195	CEACAM1	0.510	0.00004
SLC13A2	-0.916	0.00055	TMPRSS11E	0.530	0.00206	ALDH3B2	0.585	0.00004
PEG10	0.500	0.00058	ELOVL7	0.633	0.00214	CNTNAP2	-0.739	0.00004
SLC26A9	0.992	0.00059	CLEC2B	-0.537	0.00223	EDIL3	-0.524	0.00004
PLCH1	-0.525	0.00060	DUSP5	0.802	0.00227	G6PD	0.631	0.00004
CDC25B	0.524	0.00060	SLC27A2	0.517	0.00230	SYK	0.544	0.00005
UPP1	1.029	0.00063	SLC22A17	-0.597	0.00236	CLIC5	-0.724	0.00005
RHCG	0.777	0.00063	CEACAM5	0.795	0.00238	HOPX	1.177	0.00005
HLA-G	0.577	0.00066	MAP1A	-0.677	0.00239	BEX2	0.918	0.00005
NAALADL2	-0.611	0.00066	IDH1	0.511	0.00241	MAP1B	0.956	0.00005
KRT23	0.555	0.00069	TNFSF14	-0.851	0.00248	RNF150	-0.628	0.00005
SURF2	0.510	0.00069	FABP3	0.764	0.00249	MT-CO3	0.532	0.00005
PNLIPRP3	0.533	0.00070	SPRR2D	0.614	0.00252	HSPA9	0.577	0.00005
FA2H	0.648	0.00070	MX2	-0.738	0.00263	CHL1	-0.559	0.00006
IFRD1	1.004	0.00071	RSPH4A	-0.736	0.00266	CSTB	0.584	0.00006
RGS3	0.523	0.00074	APOBEC3B	0.648	0.00266	C8orf4	0.701	0.00007
RNASE7	0.781	0.00075	MELTF	-0.571	0.00281	RHOF	0.629	0.00007

IL19	-0.630	0.00076	OLFM4	-0.756	0.00294	CALCRL	-0.611	0.00007
COL1A2	-1.278	0.00076	SQSTM1	0.628	0.00299	CASP10	0.553	0.00007
C9orf84	-0.542	0.00079	IARS	0.608	0.00301	MT-ND2	0.534	0.00007
FOXD1	0.517	0.00079	C1orf54	0.644	0.00305	DEC1	-0.943	0.00007
SCN4B	-0.613	0.00082	FLT1	-0.722	0.00305	MMP13	-0.801	0.00007
MNS1	-0.609	0.00083	KRT6C	-0.984	0.00306	COL13A1	-0.886	0.00007
ALOX15	-0.519	0.00084	TMEM27	-0.514	0.00315	SLC3A2	0.571	0.00008
LGALS7B	0.532	0.00085	DNAAF3	-0.590	0.00321	SLCO2A1	-1.040	0.00008
SLC5A5	-0.834	0.00087	PADI1	0.507	0.00341	CYP26B1	-0.622	0.00008
LRRIQ1	-0.512	0.00087	TIMP3	-0.550	0.00343	NDOR1	0.785	0.00008
GK	0.542	0.00087	RIMKLA	-0.572	0.00343	CCL22	0.837	0.00008
DDIT3	1.483	0.00088	PIK3AP1	0.681	0.00355	FAM46B	-0.636	0.00008
C2orf81	-0.568	0.00090	CORO1A	0.574	0.00355	ATF3	0.520	0.00009
ASIC1	-0.790	0.00091	CYP3A5	0.780	0.00357	KRT4	-0.840	0.00009
MOCOS	0.833	0.00091	PLEKHG4B	-0.721	0.00358	CAV1	-0.517	0.00009
SLC26A2	-0.538	0.00091	SPEF1	-0.751	0.00369	CYP1A1	-0.834	0.00009
DNAH10	-0.536	0.00092	LAMP3	1.492	0.00375	ADAMTSL3	-0.604	0.00010
ALDH3A1	-0.626	0.00095	SDC2	-0.509	0.00376	EDN2	-0.686	0.00010
CLIC5	-0.645	0.00103	AC007336.3	-0.506	0.00388	VSIG10L	0.860	0.00011
CLDN10	0.606	0.00103	IFIT1	-0.576	0.00388	GK	0.542	0.00011
ITGA4	-0.507	0.00103	XPOT	0.561	0.00392	SLC22A18	0.537	0.00012
LARP6	0.742	0.00105	ICAM5	-0.534	0.00405	RINL	0.675	0.00012
NRIP3	0.722	0.00107	DNAH11	-0.509	0.00409	RPP25	0.660	0.00014
PLA2G3	0.766	0.00114	PHGDH	1.116	0.00418	CTH	1.014	0.00014
SLC16A12	-0.627	0.00116	ALOX15	-0.594	0.00433	PLOD2	-0.554	0.00014
NCCRP1	0.519	0.00118	GPD1	0.642	0.00435	PFKFB3	-0.504	0.00014
RWDD2A	-0.527	0.00118	CAPNS2	0.541	0.00444	HAPLN3	-0.537	0.00014
SMAD9	-0.546	0.00118	DMGDH	0.730	0.00450	SUSD4	-0.566	0.00014
DNAH3	-0.538	0.00120	ACAP1	-0.611	0.00465	AC010533.1	-0.913	0.00016
ABCA4	-0.627	0.00124	MSRB3	-0.507	0.00465	SERPINE2	-0.524	0.00016
ZNF563	-0.568	0.00125	KCNC3	-0.612	0.00466	IL34	0.810	0.00016
CLGN	1.119	0.00126	SPRR1A	0.543	0.00473	CEBPG	0.702	0.00018
ATP6V1C2	-0.540	0.00129	OLAH	0.741	0.00474	CAPN14	1.171	0.00018
EIF4EBP1	0.615	0.00134	CD164L2	-0.674	0.00474	SEPHS2	0.527	0.00019
IL1R2	1.138	0.00135	CTXN1	-0.513	0.00493	CHCHD10	0.527	0.00019
SLAMF7	0.545	0.00139	DDIT3	1.341	0.00494	PRR5L	-0.519	0.00019
ERN1	0.599	0.00140	HBEGF	0.788	0.00496	MPV17L	0.807	0.00020
MORN5	-0.748	0.00140	SBSN	0.568	0.00498	OLFM4	-0.641	0.00022
TTC26	-0.508	0.00143	COL21A1	-0.524	0.00499	LCP1	0.819	0.00022
WDR49	-0.568	0.00143	SLC7A7	0.558	0.00502	PLK3	0.579	0.00023
RELT	0.627	0.00143	COL6A3	-1.207	0.00506	NEURL1B	-0.524	0.00024
HHLA2	-0.524	0.00143	ACSS3	0.749	0.00509	ZC3H12A	0.580	0.00024
CLMP	0.586	0.00144	AL929554.1	-0.864	0.00513	TBC1D16	0.579	0.00024
LRRIQ3	-0.630	0.00144	LDLRAD1	-0.544	0.00519	SLITRK5	-0.903	0.00025

AMOT	-0.569	0.00148	MTHFD2	0.778	0.00526	OTUB2	0.559	0.00025
CFAP161	-0.552	0.00152	BEST1	0.631	0.00530	PAQR4	-0.640	0.00025
COL13A1	-0.629	0.00152	HGD	0.577	0.00550	B3GALT5	-0.851	0.00026
SLC22A4	-0.552	0.00152	SLC6A8	-0.613	0.00560	ENPP4	-0.525	0.00028
MTHFD2	1.050	0.00155	GULP1	0.534	0.00562	IDH1	0.501	0.00028
RIPOR2	-0.538	0.00158	NUPR1	0.864	0.00572	BAG2	0.803	0.00029
MT-ND6	0.792	0.00158	DUSP15	0.536	0.00579	IRAK2	0.528	0.00029
DEC1	-0.792	0.00161	GOLT1A	0.712	0.00596	DOC2B	0.668	0.00029
BBC3	0.633	0.00163	COL16A1	-0.524	0.00620	MCTP1	0.517	0.00030
SESN2	0.795	0.00164	TNFRSF9	-0.606	0.00623	HES6	0.595	0.00031
SLC22A18AS	0.665	0.00169	SLC7A11	0.872	0.00646	PPARGC1A	0.506	0.00031
TEX26	-0.524	0.00171	N4BP2L1	0.833	0.00652	ORAI1	0.547	0.00032
MMP12	-0.677	0.00177	NEK10	-0.647	0.00673	MSMB	-0.802	0.00033
UBALD2	0.564	0.00177	ANGPT1	-0.608	0.00678	AMOT	-0.578	0.00034
SLCO2A1	-0.675	0.00183	CCDC39	-0.603	0.00678	PRKAR2B	-0.612	0.00035
DYNC2H1	-0.517	0.00189	FOXJ1	-0.619	0.00698	GPCPD1	0.509	0.00035
HYDIN	-0.515	0.00193	CD4	-0.675	0.00704	NQO1	0.520	0.00035
MX2	-0.589	0.00193	SCN4B	-0.568	0.00712	ATP6V1C2	-0.645	0.00035
STRIP2	0.581	0.00193	NACAD	0.578	0.00725	ANLN	-0.509	0.00037
EPS8L1	0.502	0.00193	ACOT11	0.505	0.00728	AGR2	-0.545	0.00038
RELL2	0.614	0.00195	LRRC10B	-0.682	0.00733	SMOC1	0.747	0.00038
THEMIS2	0.589	0.00213	UPP1	0.793	0.00742	KCNK5	-0.597	0.00039
MEP1A	-0.945	0.00215	SLC30A2	0.617	0.00746	SCNN1G	-0.554	0.00040
ATP13A4	0.586	0.00217	GARS	0.611	0.00748	AC007336.3	-0.550	0.00041
STC2	1.391	0.00220	AL928654.5	0.595	0.00750	SPRR2A	0.903	0.00044
CEBPG	0.587	0.00225	SLITRK5	-0.654	0.00755	HSPA2	-0.610	0.00045
C2orf81	-0.535	0.00227	B3GNT4	-0.561	0.00770	ATG2A	0.647	0.00045
SERPINB2	0.518	0.00228	GDF15	1.222	0.00770	FBXO10	0.637	0.00045
KIF21B	0.720	0.00234	IGFBP5	-0.658	0.00771	TGM5	-0.600	0.00046
ALDH1L2	1.315	0.00241	RSPH1	-0.585	0.00779	CLCN4	0.523	0.00046
MAP1A	-0.602	0.00245	TOP2A	-0.543	0.00794	TMEM140	0.697	0.00046
AIFM2	0.529	0.00248	FGF1	-0.512	0.00863	SLC16A5	0.534	0.00047
IQUB	-0.518	0.00252	PLA2G2A	-0.621	0.00884	GATM	0.648	0.00048
IRAK2	0.751	0.00253	ROPN1L	-0.591	0.00909	MTHFD1L	0.536	0.00048
CXCL6	0.547	0.00255	SLC13A2	-0.535	0.00915	ABCC3	0.532	0.00054
CPT1B	0.513	0.00256	CHAC1	0.946	0.00941	FOSB	-0.719	0.00054
LTF	-0.643	0.00257	CAPS	-0.522	0.00949	FAP	-0.723	0.00056
PLIN4	0.647	0.00261	FAM129A	0.662	0.00951	SLC16A13	0.563	0.00058
EREG	0.711	0.00263	LRRC34	-0.593	0.00965	NHSL1	0.537	0.00059
RSPH14	-0.547	0.00266	SLCO4A1	0.711	0.00969	ANKRD37	-0.616	0.00060
SRD5A2	-0.666	0.00268	TLN2	0.881	0.00972	PLA2G4F	0.612	0.00062
SERPINE1	-0.619	0.00270	COL3A1	-0.963	0.00985	ID2	-0.737	0.00065
OVOL1	0.588	0.00270	KANK4	-0.663	0.00986	SYT8	-0.528	0.00065
ARHGAP20	-0.673	0.00286	SLC2A14	-0.629	0.00986	INHBB	0.580	0.00066

APOBEC3B	0.588	0.00295	ALOXE3	0.501	0.00994	DGAT2	0.553	0.00067
MAP3K19	-0.506	0.00296				CIITA	0.640	0.00067
ATP6V0D2	0.633	0.00305				MLYCD	0.516	0.00067
MSRB3	-0.567	0.00306				ABCA1	0.607	0.00070
IL33	0.639	0.00308				GPC6	-0.584	0.00071
CDC20B	-0.503	0.00317				OASL	1.001	0.00073
EBI3	0.693	0.00322				GPD1	0.760	0.00077
SLC6A9	0.826	0.00326				DGKD	0.536	0.00078
FAM71E1	0.527	0.00329				PSEN2	0.552	0.00081
ANGPT1	-0.695	0.00330				ACOT11	0.629	0.00083
C6	-0.581	0.00331				PIGM	0.537	0.00086
RNF150	-0.530	0.00337				FOLH1	-0.709	0.00086
GHR	-0.512	0.00343				ADAMTS15	-0.543	0.00088
SAMD15	-0.518	0.00345				MATN2	-0.527	0.00088
PLEKHB1	-0.518	0.00347				PTPRB	-0.525	0.00089
GPR132	0.687	0.00358				CLK1	-0.583	0.00093
KIAA2012	-0.530	0.00358				PDGFD	-0.567	0.00094
TMEM171	0.517	0.00362				MAT2A	0.526	0.00096
CFAP47	-0.584	0.00363				SLAMF7	0.689	0.00101
LYPD1	-0.648	0.00371				SLC30A2	0.765	0.00111
PTGER2	-0.601	0.00374				ABCA12	0.501	0.00112
KIF20A	-0.527	0.00375				CSPG4	-0.552	0.00112
FAM151B	-0.630	0.00375				ITGA4	-0.679	0.00113
OASL	0.863	0.00385				CUX2	-0.683	0.00113
S100P	0.955	0.00390				NDNF	-0.616	0.00113
CORO1A	0.718	0.00391				PRSS3	0.718	0.00114
SULT1A2	-0.603	0.00395				FLNC	1.147	0.00119
IFIT1	-0.506	0.00397				ANKRD22	0.629	0.00122
CFAP54	-0.563	0.00398				CXCL14	-0.745	0.00124
ZBTB16	-0.666	0.00398				HSD11B1	0.570	0.00124
ETV4	0.529	0.00399				NDRG4	0.633	0.00128
SPP1	0.653	0.00401				EDA2R	0.526	0.00134
MCM6	-0.567	0.00410				MFAP2	-0.503	0.00141
TLN2	0.690	0.00415				DUSP15	0.737	0.00142
SPINK1	0.685	0.00439				KIAA1324	-0.557	0.00142
CRNN	0.757	0.00439				CXCL3	0.558	0.00142
COL1A1	-0.643	0.00442				E2F8	-0.665	0.00144
ERICH3	-0.604	0.00464				OXNAD1	0.527	0.00144
CYP2A13	0.586	0.00473				PIK3R3	0.641	0.00145
PLA2G4C	0.653	0.00482				SAMD10	0.528	0.00145
CCNE1	0.633	0.00483				CHRNA1	0.502	0.00154
S1PR4	-0.513	0.00484				DPH7	0.578	0.00155
PRUNE2	-0.606	0.00486				LRRC37B	0.563	0.00161
MXRA5	-0.517	0.00487				PPP2R2C	0.635	0.00165

DMGDH	0.851	0.00517
SLC7A11	0.930	0.00521
CCL5	-0.508	0.00525
COL4A1	-0.713	0.00545
PPP1R3C	-0.612	0.00562
FAM110B	0.720	0.00566
ANKFN1	-0.643	0.00568
NMNAT2	-0.545	0.00582
DCDC2B	-0.634	0.00586
SLC15A1	0.512	0.00595
CHAC2	0.577	0.00599
FLI1	-0.605	0.00609
CLDN8	-0.530	0.00611
KLHL6	-0.504	0.00615
UGT2A1	-0.669	0.00620
CASC1	-0.517	0.00623
KRT78	0.740	0.00628
HBEGF	0.677	0.00676
PLA2G4B	0.576	0.00681
CEACAM7	0.511	0.00689
NRARP	0.771	0.00702
HNMT	0.503	0.00707
SLC25A25	0.714	0.00713
STK32A	0.633	0.00735
FLNC	0.700	0.00736
RCN3	-0.532	0.00744
C2CD4C	0.512	0.00756
CBX2	0.531	0.00762
APOBEC4	-0.581	0.00768
HSD17B3	-0.664	0.00772
KCNK5	-0.545	0.00775
CCL20	0.695	0.00786
PZP	-0.730	0.00799
MMP24	-0.607	0.00802
CACNB4	-0.580	0.00802
RIPOR3	0.561	0.00806
OXTR	-0.514	0.00809
RASSF2	-0.588	0.00830
SPRED3	0.553	0.00834
THSD7A	0.641	0.00845
SOWAHA	-0.535	0.00891
SCG3	-0.569	0.00892
RNF39	0.569	0.00922
CDKN2C	-0.613	0.00926

FIBIN	0.622	0.00167
FAM46C	-0.601	0.00171
PADI1	0.502	0.00171
SHH	0.669	0.00175
STXBP6	-0.778	0.00176
KLK5	1.176	0.00186
FYN	0.519	0.00186
ARHGAP20	-0.690	0.00188
SLC7A7	0.588	0.00191
GYPC	0.661	0.00191
PPFIA3	-0.586	0.00191
FAXDC2	-0.588	0.00192
TSEN15	0.506	0.00194
MYO3B	-0.732	0.00195
PPARGC1B	0.548	0.00200
TICAM2	-0.503	0.00202
CYSRT1	0.707	0.00203
ADAM12	-0.515	0.00204
MAP4K2	0.537	0.00207
ATAD3B	0.640	0.00217
TMEM14A	-0.504	0.00226
HRK	-0.743	0.00234
ARNT2	0.619	0.00236
BUB1	-0.645	0.00241
S1PR3	-0.551	0.00241
CENPV	0.623	0.00244
CD6	0.756	0.00248
MYB	-0.589	0.00253
MT-ND6	0.740	0.00263
SLC13A5	-0.561	0.00264
ZEB2	-0.711	0.00264
MAMDC4	0.512	0.00270
ADGRL3	-0.575	0.00285
TLL1	-0.571	0.00294
KIF20A	-0.647	0.00298
SCLY	0.519	0.00299
MROH6	0.507	0.00299
FGF1	-0.575	0.00304
MMP9	-0.526	0.00309
CIART	0.635	0.00311
ALOXE3	0.695	0.00316
PIK3AP1	0.604	0.00331
BCL2	0.523	0.00339
ISLR2	-0.736	0.00354

MAL	0.867	0.00940
PLAU	0.529	0.00947
ACSS3	0.568	0.00951

PMS2	0.528	0.00359
ETV5	0.756	0.00365
ZBED3	0.627	0.00367
IL11	-0.541	0.00382
CHST7	-0.599	0.00385
GJA9	-0.605	0.00398
SPRR2D	0.792	0.00418
XDH	0.523	0.00424
P2RY6	-0.665	0.00444
AC018512.1	0.590	0.00448
CYP2R1	0.511	0.00459
ATP6V1B1	-0.545	0.00466
ELL3	0.587	0.00484
ETV4	0.589	0.00489
RCN3	-0.663	0.00498
SPINK8	0.673	0.00512
GPAT3	0.651	0.00513
NFE2	-0.588	0.00520
BPIFB2	-0.528	0.00521
ATP6V0D2	0.546	0.00525
NOL9	0.514	0.00529
KLK7	0.566	0.00530
ITGB7	0.625	0.00536
AL358075.4	0.535	0.00547
SDSL	0.550	0.00557
HR	0.536	0.00584
WDR74	0.652	0.00589
RPRM	-0.676	0.00622
TYMS	-0.547	0.00627
COMP	-0.609	0.00660
NNMT	-0.639	0.00666
SLC22A18AS	0.604	0.00670
FLG	1.015	0.00684
FBP1	0.582	0.00688
SLC16A12	-0.566	0.00693
PPP2R2B	-0.664	0.00702
LMO3	-0.524	0.00704
ADGRB1	-0.538	0.00738
COL5A3	-0.636	0.00753
NPL	0.512	0.00754
SBSN	0.526	0.00762
AC009119.2	0.611	0.00770
HGD	0.585	0.00776
PLA2G4C	0.602	0.00782

	ABHD12B	0.569	0.00788
	SLC28A3	0.586	0.00810
	IL2RG	0.546	0.00813
	SULT1B1	-0.573	0.00831
	CYP20A1	0.504	0.00842
	CBX2	0.613	0.00852
	LMCD1	-0.551	0.00852
	PLA2G2A	-0.626	0.00906
	CXCL10	-0.543	0.00949
	FP565324.1	0.646	0.00952
	KRT40	-0.568	0.00972
	PDE11A	-0.502	0.00973
	BATF	0.512	0.00994

Table E3. Common DEGs shared by Control group, Asthma group and COPD group in response to laundry detergent.

Gene name	Control		Asthma		COPD	
	Log ₂ (fold change)	p-Value	Log ₂ (fold change)	p-Value	Log ₂ (fold change)	p-Value
AARS	0.969	0.00009	0.678	0.00146	1.516	0.00000
ABCC3	0.717	0.00001	0.563	0.00063	0.532	0.00054
ADM2	1.368	0.00043	1.424	0.00020	1.938	0.00000
ALDH1L2	1.315	0.00241	1.280	0.00069	2.000	0.00000
ARHGAP20	-0.673	0.00286	-0.588	0.00104	-0.690	0.00188
ASNS	1.997	0.00053	1.877	0.00005	3.061	0.00000
ATP6V0D2	0.633	0.00305	0.942	0.00001	0.546	0.00525
BEST1	1.294	0.00002	0.631	0.00530	1.539	0.00000
C6orf223	0.787	0.00000	0.568	0.00034	0.922	0.00000
C8orf4	0.767	0.00006	0.562	0.00022	0.701	0.00007
CAPN14	1.791	0.00000	1.223	0.00006	1.171	0.00018
CAT	0.553	0.00000	0.682	0.00005	0.606	0.00001
CEACAM1	0.682	0.00001	0.677	0.00037	0.510	0.00004
CHAC1	1.679	0.00003	0.946	0.00941	2.117	0.00000
CLGN	1.119	0.00126	1.481	0.00026	1.227	0.00000
CLIC5	-0.645	0.00103	-0.648	0.00030	-0.724	0.00005
COL1A1	-0.643	0.00442	-0.997	0.00097	-1.035	0.00000
COL1A2	-1.278	0.00076	-1.510	0.00004	-1.983	0.00000
COL4A1	-0.713	0.00545	-0.693	0.00011	-1.025	0.00000
COL5A1	-0.935	0.00041	-1.088	0.00008	-1.126	0.00001
CPT1A	1.357	0.00000	1.163	0.00000	1.427	0.00000
CRCT1	1.730	0.00002	1.392	0.00000	2.009	0.00000

CTGF	-0.727	0.00000	-0.682	0.00090	-0.606	0.00001
DDIT3	1.483	0.00088	1.341	0.00494	1.633	0.00000
DMGDH	0.851	0.00517	0.730	0.00450	1.701	0.00000
ECH1	0.938	0.00000	0.655	0.00001	0.991	0.00000
FAM129A	0.713	0.00041	0.662	0.00951	1.366	0.00000
GAB2	0.567	0.00013	0.543	0.00068	0.787	0.00000
GARS	0.849	0.00021	0.611	0.00748	1.114	0.00000
GDF15	1.548	0.00025	1.222	0.00770	1.112	0.00000
GK	0.542	0.00087	0.769	0.00007	0.542	0.00011
GRPEL2	0.815	0.00012	0.987	0.00048	0.569	0.00002
HBEGF	0.677	0.00676	0.788	0.00496	0.816	0.00000
HEPHL1	2.113	0.00000	1.486	0.00007	1.623	0.00000
HSD11B1	1.107	0.00003	0.813	0.00137	0.570	0.00124
IARS	0.756	0.00012	0.608	0.00301	1.069	0.00000
IFRD1	1.004	0.00071	0.866	0.00010	1.484	0.00000
IL1R2	1.138	0.00135	0.932	0.00004	0.966	0.00001
IMPA2	0.828	0.00001	0.565	0.00085	0.929	0.00000
KIF21B	0.720	0.00234	0.822	0.00076	1.391	0.00000
LAMP3	1.696	0.00040	1.492	0.00375	2.369	0.00000
LCP1	0.988	0.00001	1.248	0.00015	0.819	0.00022
LPCAT3	0.550	0.00000	0.561	0.00006	0.575	0.00001
MARS	0.848	0.00001	0.556	0.00117	1.227	0.00000
MB	-0.618	0.00002	-0.647	0.00036	-0.758	0.00000
ME1	0.900	0.00001	0.885	0.00006	1.148	0.00000
MFS4A	0.646	0.00001	0.528	0.00049	0.792	0.00000
MOCOS	0.833	0.00091	0.884	0.00081	1.592	0.00000
MT-ATP6	0.685	0.00044	0.521	0.00041	0.704	0.00000
MTHFD2	1.050	0.00155	0.778	0.00526	1.650	0.00000
NEDD9	-0.559	0.00006	-0.559	0.00001	-0.543	0.00001
NUPR1	1.218	0.00006	0.864	0.00572	1.332	0.00000
PCK2	1.109	0.00014	1.027	0.00057	1.649	0.00000
PDK4	1.378	0.00000	1.611	0.00000	2.002	0.00000
PHGDH	1.954	0.00000	1.116	0.00418	2.448	0.00000
PLIN2	2.016	0.00000	1.892	0.00002	2.139	0.00000
PRSS3	1.019	0.00001	0.748	0.00003	0.718	0.00114
PSAT1	2.103	0.00008	1.816	0.00006	2.738	0.00000
RCAN1	0.798	0.00002	0.609	0.00113	0.902	0.00000
RNASE7	0.781	0.00075	0.892	0.00074	1.025	0.00000
S100P	0.955	0.00390	0.962	0.00023	0.858	0.00000
SBSN	0.751	0.00025	0.568	0.00498	0.526	0.00762
SERPINE1	-0.619	0.00270	-0.659	0.00005	-0.909	0.00000
SLC25A20	0.531	0.00013	0.705	0.00015	0.747	0.00001
SLC28A3	0.534	0.00053	0.692	0.00016	0.586	0.00810
SLC7A11	0.930	0.00521	0.872	0.00646	1.530	0.00000

SPRR1A	1.407	0.00000	0.543	0.00473	1.184	0.00000
SPRR1B	0.821	0.00000	0.536	0.00126	0.844	0.00000
SPRR3	1.359	0.00000	1.331	0.00004	1.186	0.00001
SQSTM1	0.687	0.00000	0.628	0.00299	0.656	0.00000
SYK	0.615	0.00000	0.712	0.00022	0.544	0.00005
SYT8	-0.502	0.00026	-0.814	0.00011	-0.528	0.00065
TGFB2	-0.519	0.00000	-0.531	0.00002	-0.711	0.00000
TLN2	0.690	0.00415	0.881	0.00972	1.130	0.00004
TM6SF1	1.654	0.00000	1.588	0.00000	1.945	0.00000
TNC	-0.770	0.00005	-0.659	0.00026	-0.780	0.00000
TRIB3	2.108	0.00019	1.680	0.00186	2.681	0.00000
UPP1	1.029	0.00063	0.793	0.00742	1.159	0.00000
VNN3	1.029	0.00005	0.667	0.00060	1.296	0.00000
WARS	0.979	0.00002	0.673	0.00134	1.418	0.00000
XPOT	0.641	0.00036	0.561	0.00392	0.952	0.00000
