Group Urges Statewide Vaping Ban Amid Pandemic

spectrumlocalnews.com/nys/central-ny/ny-state-of-politics/2020/03/23/group-urges-statewide-vaping-ban-amid-pandemic

STATE OF POLITICS

By Nick Reisman New York State
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A statewide medical group on Sunday called for a complete ban on the sale of vaping products, arguing the use of e-cigarettes and similar devices will help spread coronavirus in New York.

The group, the New York State Academy of Family Physicians, urged Governor Andrew Cuomo to issue an executive order banning the sale of vaping products in New York during the crisis.

"As our state and country struggle to respond to the rapidly evolving and escalating COVID-19 pandemic affecting our residents and straining our healthcare system, mounting evidence demonstrates the link between tobacco use and increased risk for progressive COVID-19," said Dr. Barbara Keber, the group's president.

New York had already moved toward a ban on flavored vaping products amid health concerns that arose last year. State lawmakers were expected to take further action in the budget, which is due to be approved next week.

The group pointed to studies that found linkages between e-cigarette usage or smoking and the elevated risk of contracting the virus.

"People with decreased lung function caused by smoking or vaping are more likely to

develop serious complications caused by infections," Keber said.

"Now more than ever, it is critical for the State and medical community to take actions to prevent our youth from ever using these highly addictive deadly products and to help our patients to reduce their risks through FDA-approved cessation and telehealth during this pandemic."

Experts: Vaping Could Make Coronavirus Infection More Severe

futurism.com/neoscope/experts-vaping-could-make-coronavirus-infection-more-severe

Scientists say it's reasonable to assume that smoking or vaping could make COVID-19 symptoms more severe once infected, according to *Scientific American*.

To be clear, a direct link has yet to be investigated by researchers — but there's plenty of evidence that smoking or vaping suppress immune function in the lungs and trigger inflammation.

Scientists have also found that more severe COVID-19 cases were associated with chronic lung conditions — which in turn is linked to smokers and vapers as well. Some preliminary studies in China have found links between more severe COVID-19 cases and a history of smoking, but it's too early to draw conclusions as many of them still await peer review.

"All these things make me believe that we are going to have more severe cases—especially [in] people who are [long-term] smokers or vapers," said Melodi Pirzada, chief of pediatric pulmonology at NYU Winthrop Hospital on Long Island, to *Scientific American*.

"There's a very coordinated series of events that take place when you do become infected with a virus," associate professor of microbiology and immunology at the University of North Carolina Ray Pickles told *Scientific American*. "I think once you start perturbing this sequence of events in any which way or direction, that's when things can go awry."

Scientists have found plenty of evidence for smoking being a risk factor for influenza. The link to vaping, however, is definitely less clear on the matter. Mice studies have found a link between e-cigarette aerosol lowering the chances of surviving influenza A, a common influenza virus.

Health experts warn smoking, vaping could affect impact of coronavirus

wsbtv.com/news/georgia/health-experts-warn-smoking-vaping-could-affect-impact-coronavirus/NGBEPRROYNFFNESJAFCPKI6OUM

By: Samantha Manning Updated: March 24, 2020 - 8:09 PM

WASHINGTON — Health experts are warning that people who vape or smoke could face a greater threat than nonsmokers from the coronavirus.

“Because it attacks the lungs, the coronavirus that causes COVID-19 could be an especially serious threat to those who smoke tobacco or marijuana or who vape,” wrote Dr. Nora Volkow, with The National Institute on Drug Abuse.

“Thus far, deaths and serious illness from COVID-19 seem concentrated among those who are older and who have underlying health issues, such as diabetes, cancer, and respiratory conditions. It is therefore reasonable to be concerned that compromised lung function or lung disease related to smoking history, such as chronic obstructive pulmonary disease (COPD), could put people at risk for serious complications of COVID-19,” she wrote.

Anti-tobacco advocates are urging people to quit in the wake of the pandemic.

"The coronavirus is in fact a lung disease,” said Matthew Myers, president of the Campaign for Tobacco Free Kids. “Anything that weakens your lungs or immune system puts you at greater risk. If you get it, it makes it more likely you will get it more severely and have a harder time getting through it. If you're a smoker or a vaper, this is the time to quit."

The Centers for Disease Control and Prevention has not specifically placed smokers or vapers in the high-risk category for being seriously impacted by the coronavirus.

So far, it lists the elderly and people with underlying health conditions, which does include chronic lung disease.

Experts said there isn’t enough research or evidence right now to show whether there is a direct link between people who vape and people who get the coronavirus.

Research on the effects of smoking and vaping and the coronavirus is ongoing.

Vaping advocates blasted the suggestion that there might be a connection between vaping and the impact of the coronavirus.

“Even during a pandemic, activists and government bureaucrats are willing to risk their credibility by trying to tie nicotine vaping products to COVID-19,” said Gregory Conley, president of the American Vaping Association. “Lung injuries and deaths that occurred

last year were caused by illicit, contaminated THC products, not nicotine vaping products, so during this pandemic, it is important that cannabis consumers stay away from street-bought vape pens. However, adult smokers should not be scared away from using these smoke-free products by misplaced fears being pushed by those with no care or regard for adults desperately seeking to quit smoking."

Smokers At Higher Risk Of Severe COVID-19 During Coronavirus Outbreak

forbes.com/sites/victoriaforster/2020/03/23/smokers-at-higher-risk-of-severe--covid-19-during-coronavirus-outbreak

March 23, 2020

A leading expert has warned that smokers are likely at increased risk of more severe COVID-19, compared to non-smokers, suggesting that now would be a particularly good time to try and quit or cut down.

"There's not very much data at this point on COVID-19 in smokers, but we do know from reports from China, smokers seem to be over-represented in groups of people who have severe or critical COVID-19," said J. Taylor Hays, M.D. Director of the Nicotine Dependence center at Mayo Clinic in Rochester, Minnesota and Professor of Medicine, Mayo Clinic College of Medicine.

Increasing evidence is suggesting that smokers are at higher risk of severe COVID-19 than those who don't smoke. One study published in the *New England Journal of Medicine* in February looked at 1,099 patients in China with COVID-19, showing that of 173 patients who had severe symptoms, 16.9% of them were current smokers and 5.2% had previously smoked. Among the patients with less-severe symptoms, 11.8% were current smokers and 1.3% former smokers.

Today In: Healthcare

More worryingly, the study showed that in a group of patients that either needed mechanical ventilation, admission to an intensive care unit, or ultimately died, 25.5% were current smokers, which was more than twice the rate of current smokers in a group of patients that did not have these severe adverse outcomes.

"These observations about more severe illness in smokers vs people who have never smoked seems to parallel what is seen in respiratory viruses such as respiratory syncytial virus and seasonal influenza, where smokers tend to do worse than non-smokers," said Hays, also mentioning that no data is currently available on people who vape or use e-cigarettes. "We know that inhalation of combustible tobacco of any sort seems to be associated with more severe disease from respiratory viruses," he added.

Studying other coronavirus outbreaks provides further suggestions that smokers may fare worse with these types of viral infections than non-smokers. In a study of a small number of patients with Middle-East Respiratory Syndrome (MERS) in South Korea, patients who smoked were less likely to survive than those who did not. There was also some evidence that smokers had higher levels of a protein called DPP4, a receptor which allows the MERS coronavirus to enter cells in the lung, which could make their lung cells

more susceptible to attack from the virus. SARS-CoV2, the coronavirus responsible for the current outbreak, uses a different receptor to gain access to lung cells called ACE2. However, the news here isn't any better for smokers either.

"The ACE2 receptor is up-regulated in the respiratory cells of smokers. This might be a mechanism by which it is more likely to cause severe illness," said Hays.

There are also other, well-proven reasons for smokers to be concerned about their risk of severe or fatal COVID-19.

"There is a long history of smokers having more severe respiratory illness in general and this is for a few well-established reasons. They clear mucus less efficiently, the cilia which get infectious particles and secretions out of the lungs, work less efficiently. Smoking also causes inflammation in the airways, which is made worse with respiratory illnesses," said Hays.

So if you are reading this and you smoke or know someone who does, is it too late now to stop or cut down?

"People who quit for even a short time see an improvement in lung health quite quickly. For most smokers who don't already have serious lung injury, they will see immediate improvements in their health, and less opportunity for severe diseases including COVID-19," said Hays.

In 2015, the CDC reported that almost 7 out of 10 adult smokers wanted to try and quit, with over half of all of them trying to quit at least once in that year, but the large majority not succeeding. Is it likely that people will try to quit, and succeed, especially at such a stressful time for many?

"I understand people turn to things because it's a coping mechanism, especially at stressful times. I would say to them - try and flex other coping muscles, there is a real opportunity to break routines – even a short period of abstinence from smoking improves lung function," said Hays.

"People could look at this as an opportunity – a time of crisis is a time of opportunity. If you've been looking for an opportunity to quit, this is it," he added.

Victoria Forster
I am a postdoctoral research scientist focusing on childhood cancers and new, targeted cancer therapies. As a survivor of childhood leukemia myself, I am a determined

...

Coronavirus concerns: Smoking and vaping risk

turnto10.com/features/health-landing-page/coronavirus-concerns-smoking-and-vaping

People who smoke or vape are considered at high risk for complications of COVID-19, according to the Rhode Island Department of Health.

Dr. Doug Martin, who is a pulmonologist at Lifespan, said while it's no secret that there are health ramifications from smoking and vaping, including the potential to damage our lungs, that risk has increased amid the coronavirus pandemic.

"What I tell my patients about smoking is, unfortunately, when you smoke and you breathe this stuff into your nose and out into your lungs, you're delivering it throughout your body and in the blood stream, and so that's why we get all these different cancers and then the injury to the lung," Martin said.

In addition to the health department, Martin said there's evidence from China that it can worsen the illness.

"They took 78 patients that had it and they looked at people that got sicker versus people that didn't, and with the people that got sick, 27 percent of them had a history of smoking," Martin said.

Smoking and vaping, he said, impacts the integrity and immune function in the lungs.

He said everyone, including young adults, need to pay attention.

"There's an emerging concern that we've seen in the U.S., where there does look to be some younger people that are getting sicker with this coronavirus than you would expect," Martin said. "There are now a large number of anecdotal reports that at least a good number of these do have a vaping or smoking history."

Martin and other health officials continue to say that hand-washing and physical distancing are two easy ways to prevent the spread.

But he said we need our lungs to be healthy to help fight this respiratory illness.

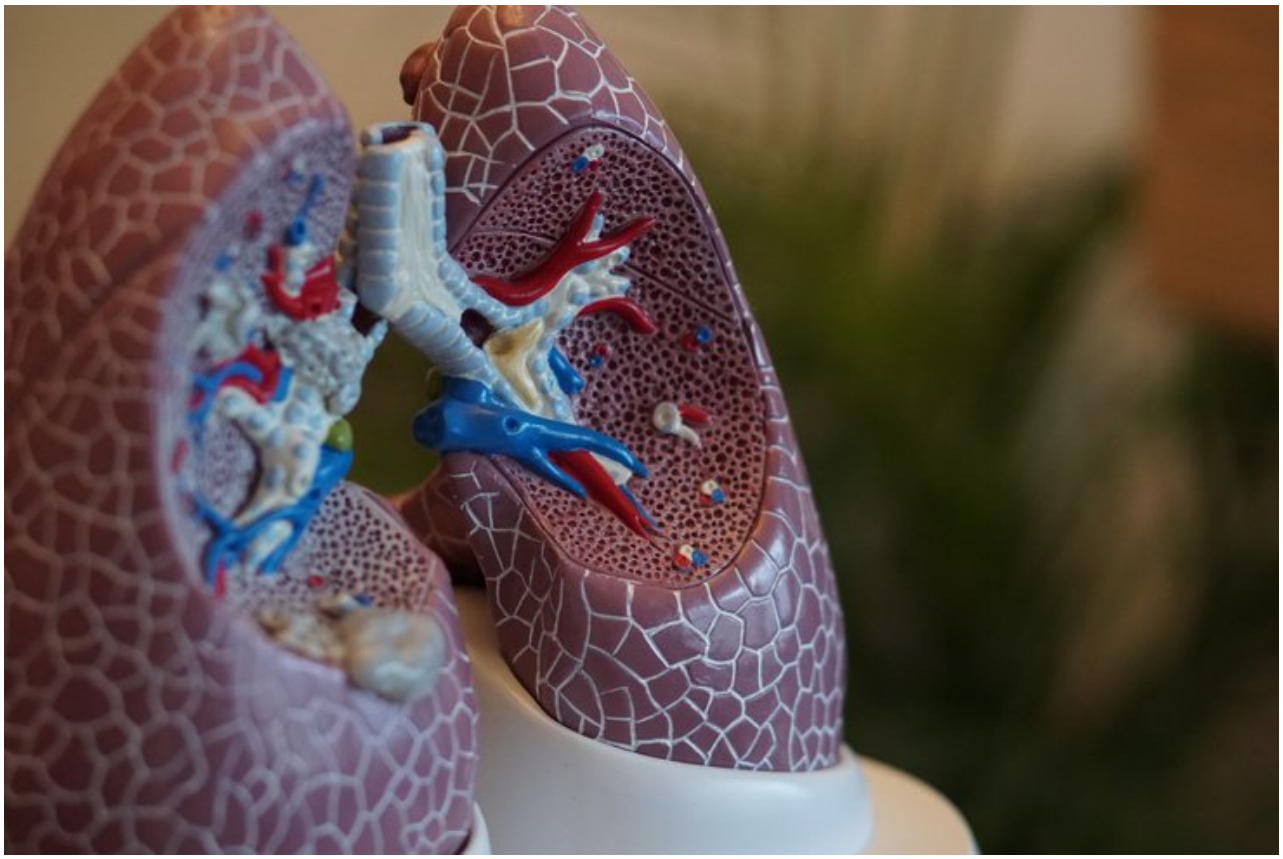
If you're looking to quit smoking or vaping, the American Heart Association has a list of resources.

Smokers are at greater risk of severe illness caused by COVID-19, researchers find

phillyvoice.com/coronavirus-smoking-risk-severe-complications-copd-covid-19

March 23, 2020

Illness Coronavirus



Smokers and people with lung diseases have a greater risk of developing severe coronavirus cases, according to research from China, where the COVID-19 pandemic originated. The virus attacks the lungs, making it particularly dangerous for people who smoke tobacco or marijuana, according to the National Institute on Drug Abuse.

As scientists compile more data on the ways COVID-19 affects the body, one thing has become clear: smokers and people living lung disease have a greater risk of developing severe illness.

Early studies out of China, where the coronavirus pandemic originated, show a higher fatality rate among people with respiratory disease. One study conducted by the Chinese Center for Disease Control found a 6.3% fatality rate among people with chronic respiratory disease compared to a 2.3% fatality rate among all patients infected with the virus.

- Digestive symptoms, diarrhea could be signs of coronavirus, Chinese study finds

Another study published in the Chinese Medical Journal found that smokers were 14 times more likely to have serious complications than nonsmokers.

Because the coronavirus attacks the lungs, it can be particularly dangerous for people who smoke tobacco or marijuana, or those who vape, according to the National Institute on Drug Abuse.

Smokers are already at greater risk for pneumonia – a complication related to COVID-19 – than the general population because the habit weakens the lung's ability to fight off infection. They are also more likely to develop chronic obstructive pulmonary disease, an umbrella term for various progressive lung diseases, including emphysema and chronic bronchitis.

A study published on the MedRxiv scientific study site helps connect the dots between smoking, COPD and COVID-19 complications.

Shortness of breath, a common characteristic of COPD and a coronavirus symptom, has been continuously linked to more severe coronavirus cases. While patients with shortness of breath were 3.7 times more likely to have severe COVID-19 than those without it, patients with COPD were 6.4 times more likely.

Vageesh Jain of University College London's Institute for Global Health, pooled data from seven smaller studies from China, analyzing data on more than 1,813 patients hospitalized with COVID-19. Those who suffered from shortness of breath, formally known as dyspnoea, tended to have more severe cases.

"Whilst dyspnoea was not a particularly common symptom in COVID-19 patients, its significant association with both severe disease and ICU admission may help clinicians discriminate between severe and non-severe COVID-19 cases," Jain said in a statement.

So what is the takeaway from all this data? Health experts are encouraging smokers to make quitting a priority. And they are urging people with lung disease to carefully monitor any changes to their health.

"It is vital to heed public health warnings on social distancing and avoiding public places when possible," The American Lung Association advises. "If you are at-risk, be attentive to any possible symptoms – fever, increased cough or shortness of breath from your baseline and be more communicative with your caregivers. Stay on your medication as directed and be careful to make sure you don't run out."

Locally, Trinity Health is instituting new measures to help smokers.

"Trinity Health has prioritized reducing tobacco use across our 22-state health system through a commitment to tobacco screening and referral connecting patients to cessation resources, and advocacy for anti-tobacco policies at the federal, state and local levels," Dr. Daniel Roth, chief clinical officer and Dr. Mouhanad Hammami, senior vice president of community health and well being, said in a statement.

The Philadelphia Department of Public Health also has resources at SmokeFreePhilly.

DPP4, the Middle East Respiratory Syndrome Coronavirus Receptor, is Upregulated in Lungs of Smokers and Chronic Obstructive Pulmonary Disease Patients

academic.oup.com/cid/article/66/1/45/4083573

Abstract

Background

Middle East respiratory syndrome coronavirus (MERS-CoV) causes pneumonia with a relatively high case fatality rate in humans. Smokers and chronic obstructive pulmonary disease (COPD) patients have been reported to be more susceptible to MERS-CoV infection. Here, we determined the expression of MERS-CoV receptor, dipeptidyl peptidase IV (DPP4), in lung tissues of smokers without airflow limitation and COPD patients in comparison to nonsmoking individuals (never-smokers).

Methods

DPP4 expression was measured in lung tissue of lung resection specimens of never-smokers, smokers without airflow limitation, COPD GOLD stage II patients and in lung explants of end-stage COPD patients. Both control subjects and COPD patients were well phenotyped and age-matched. The mRNA expression was determined using qRT-PCR and protein expression was quantified using immunohistochemistry.

Results

In smokers and subjects with COPD, both DPP4 mRNA and protein expression were significantly higher compared to never-smokers. Additionally, we found that both DPP4 mRNA and protein expression were inversely correlated with lung function and diffusing capacity parameters.

Conclusions

We provide evidence that DPP4 is upregulated in the lungs of smokers and COPD patients, which could partially explain why these individuals are more susceptible to MERS-CoV infection. These data also highlight a possible role of DPP4 in COPD pathogenesis.

Middle East Respiratory Syndrome coronavirus (MERS-CoV) is a newly emerging pathogen that mainly causes pneumonia with a relatively high case-fatality rate [1, 2]. Since 2012, ~1900 laboratory-confirmed cases have been reported to the World Health Organization (WHO) [2]. The majority of cases occurred in familial or hospital-related clusters through human-to-human transmission [3–5]. The clinical course of MERS-CoV

infection ranges from asymptomatic to acute respiratory distress syndrome with need for ventilatory support [3, 5–7]. To infect its host, MERS-CoV attaches to its receptor, an exopeptidase called dipeptidyl peptidase 4 (DPP4), also known as CD26 [8].

DPP4 is a type II transmembrane glycoprotein that is expressed in many cell types and organs in the body. It serves multiple functions among which post-translational cleavage of hormones and chemokines, T-cell activation, cell adhesion, and apoptosis [9–11]. In lungs, however, DPP4 is expressed at a minimum level [12], mainly in alveolar epithelial cells and endothelial cells, and to a lesser extent in bronchiolar epithelial cells, airway submucosal glands, alveolar macrophages, lymphocytes, and plasmacytoid dendritic cells [13–16]. Importantly, the alveolar epithelial cells are the main target for MERS-CoV [13, 17].

Several underlying comorbidities, including chronic lung diseases, have been reported to increase the risk of acquiring MERS-CoV infection [18]. Chronic obstructive pulmonary disease (COPD) is a highly prevalent chronic lung disease in older subjects and is currently the leading cause of death worldwide [19, 20]. The most common cause of COPD is chronic cigarette smoking. The inflammatory response to cigarette smoke results in an excessive release of chemokines and cytokines with a subsequent high influx of immune cells [20]. Because smoking has also been reported to increase susceptibility to MERS-CoV infection [18], we aimed to investigate the expression of the MERS-CoV receptor, DPP4, in a large well-phenotyped cohort of smokers, with and without airflow limitation, in comparison to age-matched individuals that never smoked (never-smokers).

METHODS

Human Lung Tissue Samples

Lung resection specimens were obtained from patients diagnosed with solitary pulmonary tumors at Ghent University Hospital (Ghent, Belgium) or from explant lungs from end-stage COPD patients (UZ Gasthuisberg, Leuven, Belgium). Based on preoperative spirometry, diffusion capacity tests and questionnaires, patients were categorized as never-smokers with normal lung function, smokers without airflow limitation or patients with COPD. COPD severity was defined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification [19]. None of the patients were treated with neo-adjuvants chemotherapy. Lung tissue of patients diagnosed with solitary pulmonary tumor was obtained at a maximum distance from the pulmonary lesions and without signs of retro-obstructive pneumonia or tumor invasion and collected by a pathologist. Lung tissue of patients with COPD GOLD III-IV was obtained from lung explants of end-stage COPD patients undergoing lung transplantation. Written informed consent was obtained from all subjects. This study was approved by the medical ethical committees of the Ghent University Hospital (2011/114) and the University Hospital Gasthuisberg Leuven (S51577). Patient characteristics are listed in Table 1. Detailed patient characteristics per read-out are provided in

supplementary Tables S1 and S2.

Table 1.

Characteristics of study population (n = 117)

	Never-smokers	Smokers[a]	COPD II[b]	COPD III–-IV[c]
Number	21	32	37	27
Sex (M/F)	6/15[d]	23/9[d]	34/3[d]	12/14[d]
Age (years)	65 (58–71)	64.5 (55–71)	65 (58–69)	56.5 (54–60)[e,f,g]
Current- / ex-smoker	-	19/13[d]	24/13[d]	0/27[d]
Smoking history (PY)	0 (0–0)	33 (14–51)[e]	45 (40–60)[e,f]	30 (25–36)[e,g]
FEV_1 post (L)	2,4 (2,1–3)	2,7 (2,3–3,3)	2,1 (1,8–2,4)[e,f]	0,7 (0,5–1)[e,f,g]
FEV_1 post (% predicted)	103 (92–117)	95 (92–112)	69 (61–75)[e,f]	27 (21–33)[e,f,g]
FEV_1 / FVC post (%)	78 (74–83)	76 (72–79)	56 (51–61)[e,f]	30 (27–35)[e,f,g]
DLCO (% predicted)	88 (81–103)	83 (65–104)	67 (51–86)[e,f]	34 (32–37)[e,f,g]
KCO (% predicted)	95 (86–121)	93 (78–106)	85 (65–107)[e]	52 (46–59)[e,f,g]
ICS (yes/no)	1/19[d]	2/30[d]	16/21[d]	25/1[d]

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Human Proximal Bronchi Samples

Biopsy samples of proximal bronchi were obtained from 21 patients (17 male and 4 female) with moderate-to-severe COPD previously recruited for a separate study [21]. Inclusion criteria were the following: chronic productive cough, age between 40 and 70 years, current smokers, negative skin tests for inhalation allergens, FEV_1 < 70% of

predicted normal value or $FEV_1/VC < 0.70$, reversibility of $FEV_1 < 10\%$ pred after 750 μg terbutaline inhalation, and suffering from moderate-to-severe bronchial hyper-responsiveness, as determined by PC_{20} value upon challenge with histamine and methacholine. Exclusion criteria were a history of asthma, complaints of wheezing, recent respiratory tract infection, and recent or concurrent usage of anti-inflammatory drugs. Oral anti-inflammatory medication was discontinued for at least 3 months and inhaled glucocorticoids at least 6 weeks before the start of the study. Bronchoscopy was performed with an Olympus BF 1T10. At least 6 biopsies were taken from the bronchi of the right and the left upper and lower lobes using a fenestrated forceps (FB-18C or FB-20C). All was according to published guidelines [22]. The study was approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam, and written informed consent was obtained from all participants. Patient characteristics are listed in Table 2. Proximal bronchi biopsy samples of 16 healthy individuals (8 male and 8 female), previously described in the earlier study [23], were used as negative control.

Table 2.

Characteristics of the Patients in Which Proximal Bronchi Biopsy Samples Were Obtained

	Mean ± SD	Median	Range
Age, years	56.3 ± 8.9	60	42–46
Actual smoking, cigarettes/day	15.4 ± 7.4	13	6–30
Pack-years	25.3 ± 11.2	21	5–50
FEV_1, % predicted	62.5 ± 12.9	65	34–93
Reversibility, % predicted	5.3 ± 3.1	5	0–9.0
PC_{20}, mg/ml			
For histamine	1.7 ± 2.1	0.87	0.11–8
For methacholine	4.6 ± 5.5	1.72	0.6–17.4

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Table 3 provides an overview of the different cohorts and samples used in this study.

Table 3.

Overview of the Cohorts and Samples Used in This Study

Overview of cohorts and samples used

90 patients (21 never-smokers, 32 smokers without airflow limitation and 37 patients with COPD GOLD stage II) who underwent lobectomia or pneumectomia due to lung cancer.
•73/90 patients: samples for both qRT-PCR and IHC analyses. •5/90 patients: samples only for qRT-PCR analysis. •12/90 patients: samples only for IHC analysis.
27 patients with COPD GOLD stage III–IV who underwent lung transplantation due to end-stage COPD.
•14/27 patients: samples for qRT-PCR analysis. •13/27 patients: samples for IHC analysis.
37 patients who underwent bronchial biopsies.
•21/37 patients with moderate-to-severe COPD (ref): samples used for IHC staining. •16/37 control patients with airflow limitation (ref): samples used for IHC staining.

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Purification of Human Lung Dendritic Cell-subsets

Lung dendritic cells (DC) were isolated from single cell suspensions of lung tissue of 3 patients, as described previously [24]. Lung tissues were rinsed, cut into small fragments, and incubated in digestion medium. Next, the samples were resuspended in Ca2+ and Mg2+–free PBS containing 10 mM EDTA and passed through a 40 μm filter. Subsequently, pulmonary mononuclear cells were separated on a Ficoll density gradient. The cells were labeled with anti-CD3-FITC, anti-CD19-FITC, anti-CD207-PE, anti-CD209-PerCp-Cy5 and anti-BDCA2-APC and sorted on a FACSAria (BD Biosciences).

RNA Extraction and Real-Time Polymerase Chain Reaction Analysis

RNA extraction and polymerase chain reaction (PCR) analysis of lung tissue were performed as described previously [25]. RNA extraction from lung tissue blocks of 92 subjects (18 never-smokers, 26 smokers without airflow limitation, 34 patients with COPD GOLD II, 14 patients with COPD GOLD IV) was performed with the miRNeasy Mini kit (Qiagen, Hilden, Germany), following manufacturer's instructions. Next, complementary DNA (cDNA) was prepared with the iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad, Hercules, California). Taqman Gene Expression Assays (Applied Biosystems, Forster City, California) were used to measure the expression of DPP4 and the reference genes Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Hypoxanthine phosphoribosyltransferase-1 (HPRT-1) and Succinate Dehydrogenase

complex flavoprotein subunit A (SDHA). Data were analyzed using the standard curve method, and expression of DPP4 was calculated relative to the expression of the 3 reference genes, using the geNorm applet according to the guidelines and theoretical framework previously described [25, 26].

For human lung DC subsets, RNA extraction was performed with miRNeasy Mini kit (Qiagen, Hilden, Germany), whereas RNA amplification was with the Qiagen QuantiTect Whole Transcriptome kit, both following manufacturer's instructions. DPP4 expression in the DC subsets was calculated relative to the expression of GAPDH, HPRT1 and peptidylprolyl isomerase A (PPIA) as described previously [25].

DPP4 Immunohistochemistry and Analyses

Sections obtained from formalin-fixed paraffin-embedded lung tissue blocks of 98 subjects (19 never-smokers, 30 smokers without COPD, 36 subjects with COPD GOLD II, and 13 subjects with COPD GOLD III-IV) were incubated with anti-DPP4 antibody (polyclonal goat-anti-human, R&D Systems, AF1180) [15] after antigen retrieval with citrate buffer (Klinipath, Olen, Belgium). Next, slides were colored with diaminobenzidine (Dako, Carpinteria, California) and counterstained with Mayer's hematoxylin (Sigma-Aldrich, St-Louis, Missouri). The isotype control was goat immunoglobulin G (IgG) from R&D Systems (Abingdon, UK) (AB-108-C). To co-stain DPP4 with alveolar epithelial cells, anti-aquaporin 5 (Abcam, Cambridge, UK) (ab92320) and pro-surfactant C (Abcam) (ab90716) were used to detect, respectively, type I and type II alveolar cells and subsequently colored with Vector Blue (Vector, Peterborough, UK).

Quantitative scoring of the amount of DPP4-positive scoring in alveolar tissue and airway epithelium was performed using the Axiovision software (Zeiss, Oberkochen, Germany). In order to measure the area of DPP4-positive signal in alveolar tissue, 15 images of alveolar tissue were recorded from an average of 3 tissue blocks per patient. The intensity of brown staining we wished to score was selected by means of selecting specific hue, lightness, and saturation values. The hue, saturation, and lightness values were identical for all images, therefore restricting our scoring to a specific signal. In every image the alveolar tissue was selected and the DPP4-positive signal was calculated only within the alveolar tissue and normalized to the area of alveolar tissue present in each image. The final score of each patient was the average ratio of DPP4-positive signal of the 15 images. In the airway epithelium, the amount of DPP4 signal was normalized to the length of the basement membrane (Pbm). The final score of each patient was the average DPP4 staining in all airways present in all tissue blocks available of that patient. The number of airways per patient was between 3 and 20.

DPP4 detection in the frozen samples of proximal bronchi was performed with 1 μg/mL mouse anti-DPP4 monoclonal antibody (Santa Cruz Biotechnology, Dallas, Texas) [15], after previously fixed with acetone and incubated with 10% normal goat serum (Dako, Glostrup, Denmark) for 1 hour at room temperature. These slides were subsequently stained with biotinylated goat antimouse Ig serum (1:50 in PBS/BSA plus 10% human

serum) and with streptavidin alkaline phosphatase (1:50 in PBS/BSA plus 10% human serum; Biogenex, Klinipath, Duiven, The Netherlands) for 30 minutes each. A positive signal was revealed with New Fuchsin substrate (Chroma, Kongen, Germany). Counterstaining was performed with Gill's hematoxylin. Negative control staining was performed by the substitution of the primary monoclonal antibody with an isotype antibody.

Statistical Analysis

Statistical analysis was performed with Sigma Stat software (SPSS 23.0, Chicago, Illinois), using Kruskal-Wallis, Mann-Whitney *U*, Fisher exact test, and Spearman correlation analysis. In addition, one-way analysis of variance (ANOVA) and *T*-tests were used for statistical analyses of the DC subsets. Characteristics of the study population are presented as a median and interquartile range. Differences at *P*-values < .05 were considered to be significant (**P* < .05, ***P* < .01, and *** *P* < .001).

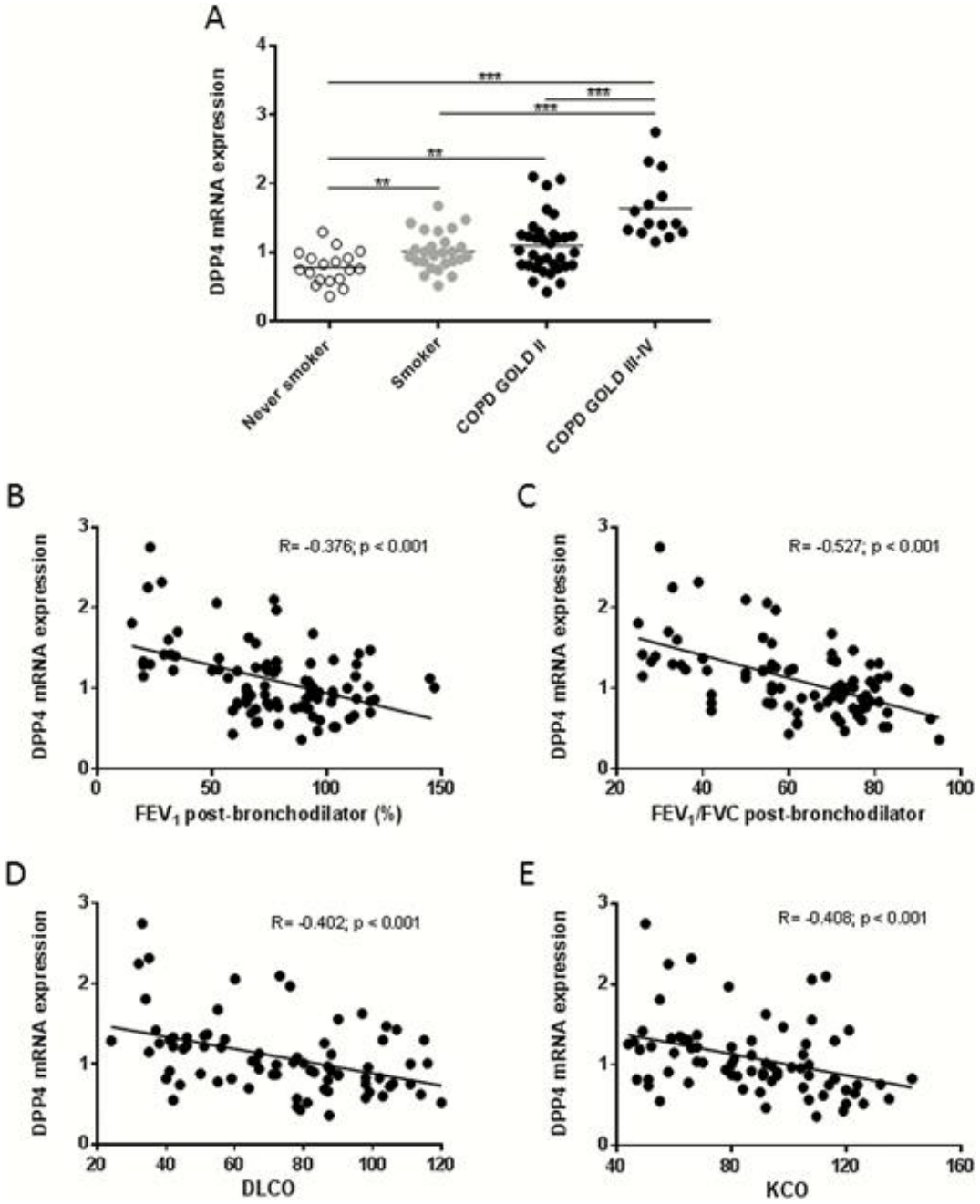
RESULTS

DPP4 mRNA Expression is Upregulated in Lungs of Smokers and COPD Patients

Messenger RNA (mRNA) expression of DPP4 was analyzed in lung tissue of 92 subjects. Lung tissue was derived from either resection tissue of lobectomy (never smokers, smokers without airflow limitation and patients with COPD GOLD stage II) or explant lungs of lung transplantation (patients with COPD GOLD stage III–IV). Patient characteristics are described in supplementary Table 1.

Compared to never-smokers, mRNA expression of DPP4 in lung tissue of smokers without airflow limitation and patients with COPD was significantly increased (Figure 1A). Moreover, DPP4 mRNA expression in lung tissue of patients with COPD GOLD stage III–IV was significantly higher than in lung tissue of smokers without airflow limitation and patients with COPD GOLD stage II (Figure 1A). Quantification according to smoking status (ex- vs. current smokers) is shown in Supplementary Figure S1. Furthermore, DPP4 mRNA expression was inversely correlated with the severity of airflow limitation: FEV_1 (R = −0.376, *P* < .001) and FEV_1/FVC ratio (R = −0.527, *P* < .001) (Figure 1B–C). In addition, the mRNA expression of DPP4 was also correlated inversely with the diffusing capacity of the lung, DLCO (R = −0.402, *P* < .001) and KCO (R = −0.408, *P* < .001) (Figure 1D–E). Linear regression analysis revealed that the association of DPP4 mRNA expression with the presence of COPD was significant even when corrected for age, sex, pack-years, and use of inhaled corticosteroids (Supplementary Table S3).

Figure 1.



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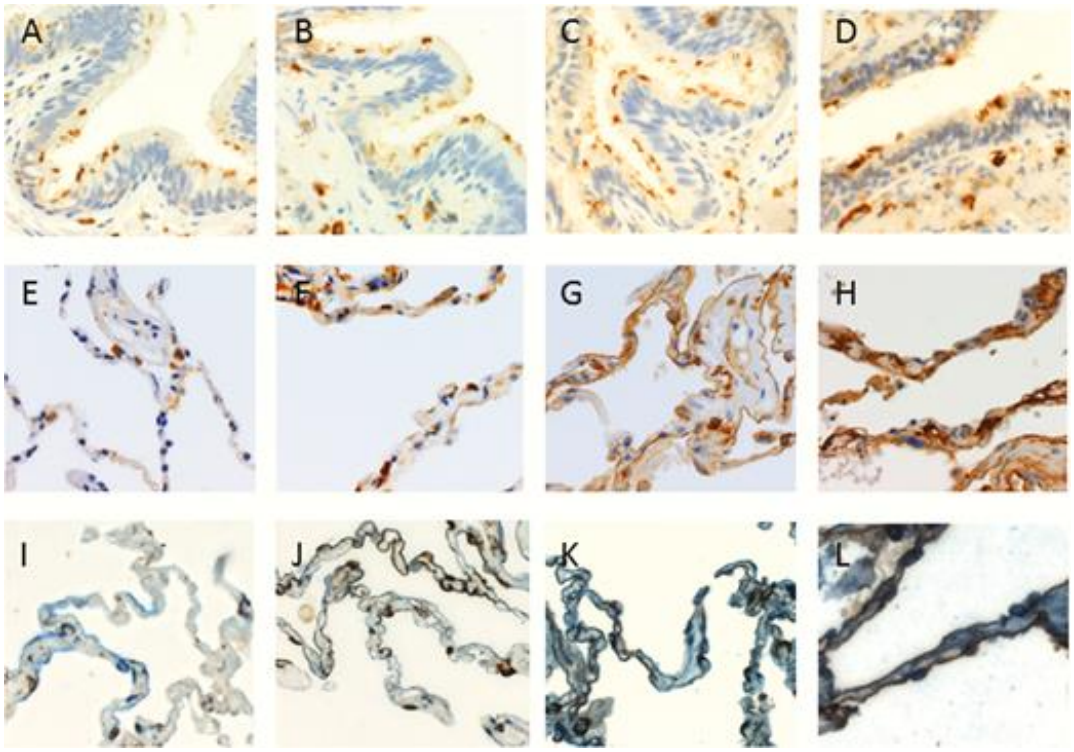
DPP4 mRNA expression in the lung tissues of smokers and COPD patients. *A*, DPP4 mRNA expression was measured by qRT-PCR and normalized to three reference genes (GAPDH, HPRT-1, SDHA). DPP4 mRNA expression in the lungs of smokers and COPD patients is significantly higher in comparison to that of never smokers. *B*, Correlation of DPP4 mRNA expression with post-bronchodilator FEV_1 values. *C*, Correlation of DPP4 mRNA expression with post-bronchodilator Tiffeneau index (FEV_1/FVC). *D*, Correlation of DPP4 mRNA expression with DLCO (diffusing capacity or transfer factor of the lung for carbon monoxide). *E*, Correlation of DPP4 mRNA expression with KCO (carbon monoxide transfer coefficient). $^{**}P < .01$, $^{***}P < .001$. Abbreviations: COPD, chronic obstructive pulmonary disease; DLCO, diffusing capacity of the lung for carbon monoxide; FEV/FVC, forced expiratory volume in 1 second/forced vital capacity; GOLD, global initiative for obstructive lung disease; KCO, transfer of carbon monoxide coefficient; mRNA, messenger RNA; qRT-PCR, quantitative reverse-transcription polymerase chain reaction.

Additionally, because dendritic cells (DCs) play a crucial role in antiviral immunity, we investigated whether DPP4 mRNA expression differs between DC subsets. Three DC subsets were sorted: langerin-positive DCs, DC-SIGN-positive DCs, and plasmacytoid DCs (pDCs). DPP4 mRNA was merely detected in pDCs (Supplementary Figure S2).

DPP4 Protein Expression is Upregulated in Lungs of Smokers and COPD Patients

DPP4 protein expression was studied in lung tissue of never-smokers, smokers without airflow limitation, and COPD patients by using immunohistochemistry (IHC) staining. DPP4 was detected on the apical surface of bronchiolar epithelium and in the alveolar epithelial cells. In the alveoli, we observed that DPP4 protein was gradually increased from never-smokers to COPD GOLD stage III–IV (Figure 2). Additionally, we performed immunohistochemical staining of DPP4 with both aquaporin 5 (marker of type I alveolar epithelial cells) and pro-surfactant C (marker of type II alveolar epithelial cells), confirming that the upregulation of DPP4 protein can mainly be contributed to the alveolar epithelial cells (Figure 2I–L). In contrast, this increment was not observed in the bronchiolar epithelium (Figure 2A), as well as in the proximal bronchial epithelium (Figure 3). Furthermore, DPP4 was also detected in the endothelial cells, alveolar macrophages, immune cells in the submucosal region of airway epithelium, and lymphoid aggregates (Supplementary Figure S3). We further quantified DPP4 signals in the lung tissues of 98 subjects using the Axiovision software (Zeiss). Characteristics of these patients are presented in supplementary Table 2.

Figure 2.



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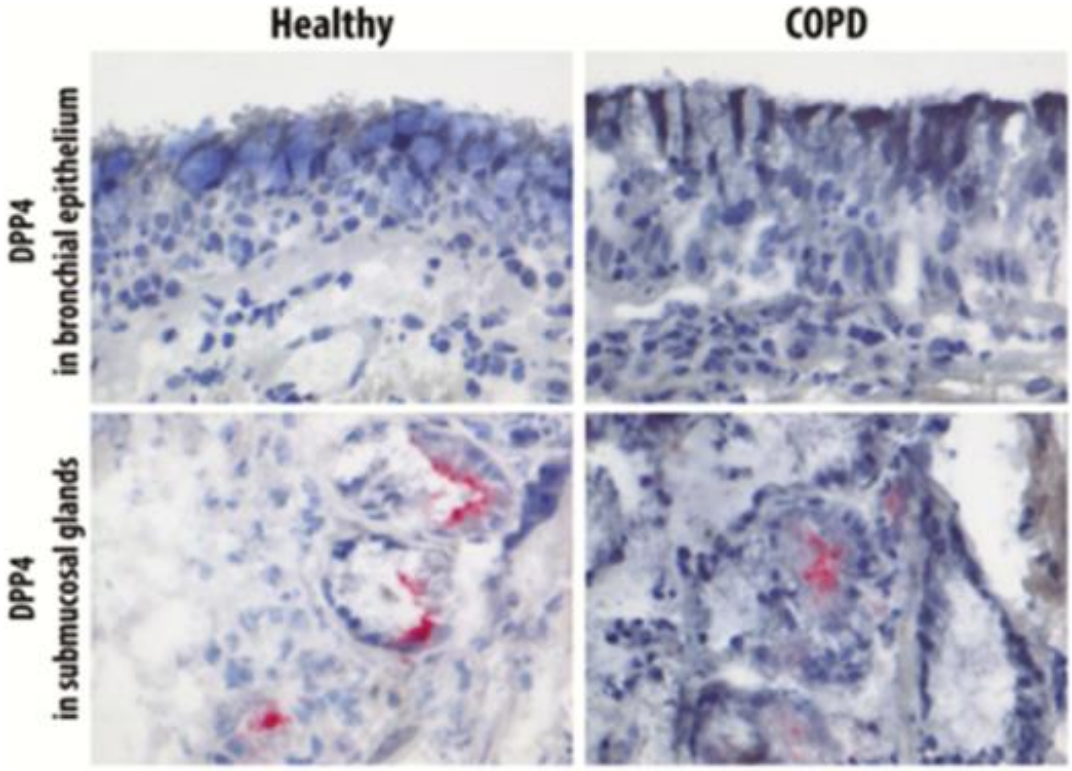
DPP4 protein expression in the bronchiolar epithelium and the alveolar tissues of never smoker, smoker, and COPD patients. Representative images of DPP4 staining in the bronchiolar epithelium (top row) and alveoli (middle and bottom row) of *A,E,I,* never-smoker, *B,F,J*, smoker without airflow limitation, *C,G,K*, subject with COPD GOLD stage II and *D,H,L*, subject with COPD GOLD stage III–IV. *I*–-*L*, are immunohistochemical stainings of DPP4 (brown) and aquaporin 5 (marker of type I alveolar epithelial cells) and pro-surfactant C (marker of type II alveolar epithelial cells) (both in blue). Co-staining of DPP4 with either one of the alveolar epithelial cell types results in a dark brown stain. DPP4 was mainly expressed in the alveolar epithelial cells and expressed the most intense in the COPD GOLD stage III–IV group. A 400× magnification was used for all photomicrographs in this figure. Abbreviation: COPD, chronic obstructive pulmonary disease; GOLD, global initiative for chronic obstructive lung disease.

Figure 3.



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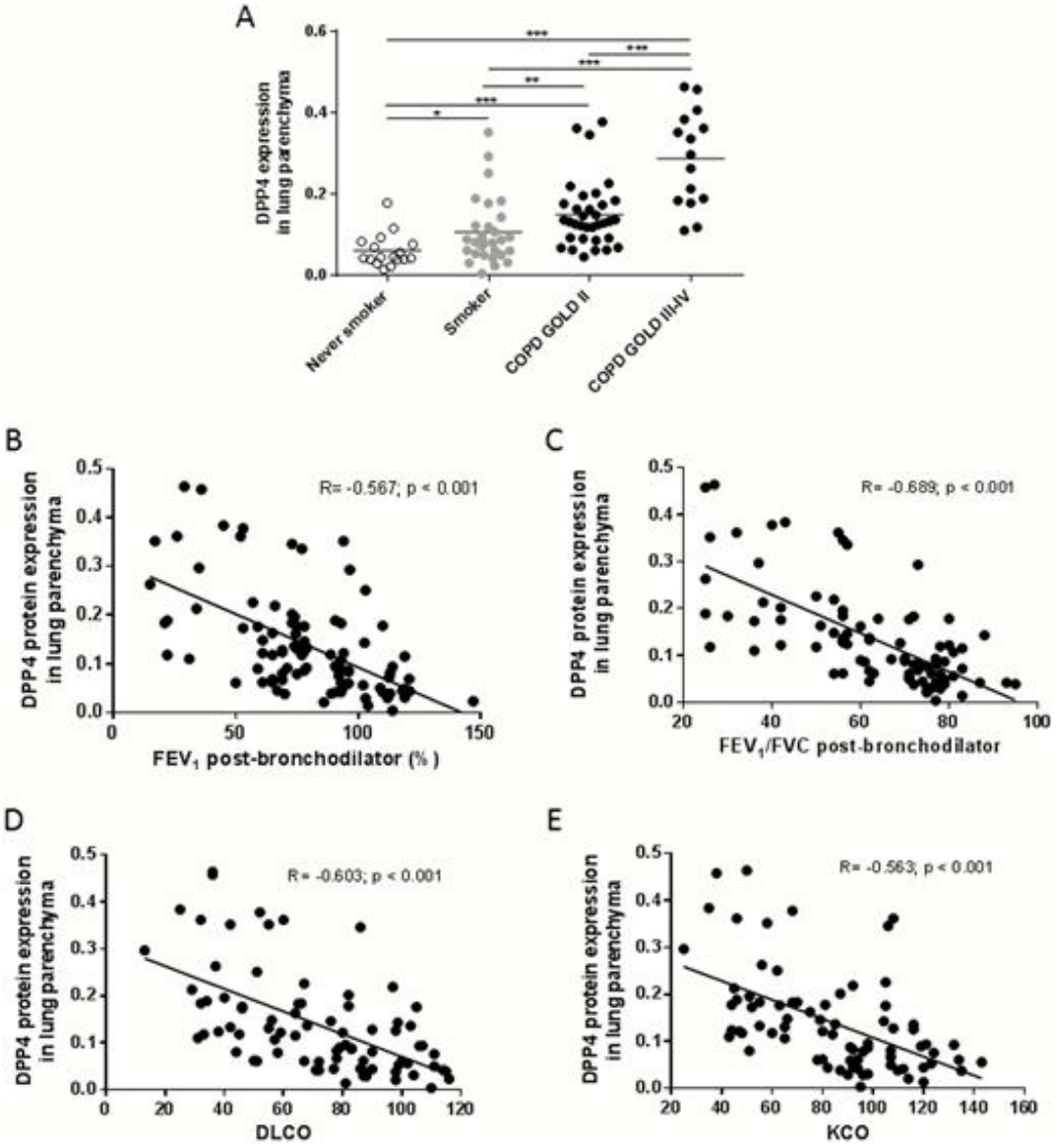
DPP4 staining in the proximal bronchi epithelium. Representative images of DPP4 staining in proximal bronchial epithelium and submucosal glands of the healthy control subject with COPD GOLD stage II. DPP4 was hardly detected in the apical surface of the proximal bronchi epithelium of both healthy control and COPD patients. Submucosal glands here served as positive control for DPP4 staining. Abbreviation: COPD, chronic obstructive pulmonary disease.

Compared to never-smokers, DPP4 protein expression was significantly increased in the alveolar epithelial cells of smokers and patients with COPD. DPP4 protein expression was the highest in patients with COPD GOLD stage III–IV (Figure 4A). Quantification of DPP4 protein expression according to smoking status (ex- vs. current smoking) is shown in

Supplementary Figure S4. Similar to DPP4 mRNA expression, DPP4 protein was also inversely correlated with lung function parameters FEV_1 (R = −0.567, $P < .001$) and FEV_1/FVC ratio (R = −0.689, $P < .001$); as well as diffusing capacity parameters DLCO (R = −0.603, $P < .001$) and KCO (R = −0.563, $P < .001$). Linear regression analysis revealed that the association of alveolar DPP4 expression with the presence of COPD was significant even when corrected for age, gender, pack-years, and use of inhaled corticosteroids (Supplementary Table S4).

Figure 4.



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DPP4 protein expression in the lung tissues of smokers and COPD patients. , DPP4 protein expression was analyzed by using Axiovision software (Zeiss). The area of DPP4 positive signal was normalized to the total area of cells present in each analyzed image. DPP4 protein expression in the lungs of smokers and COPD patients is significantly higher in comparison to that of never smokers. *B*, Correlation of alveolar DPP4 protein expression with post-bronchodilator FEV_1 values. *C*, Correlation of alveolar DPP4 protein expression with post-bronchodilator Tiffeneau index (FEV_1/FVC). *D*, Correlation of alveolar DPP4 protein expression with DLCO (diffusing capacity or transfer factor of the lung for carbon monoxide). *E*, Correlation of alveolar DPP4 protein expression with KCO (carbon monoxide transfer coefficient). $^{**}P < .01$, $^{***}P < .001$. Abbreviation: COPD, chronic obstructive pulmonary disease.

DISCUSSION

Our study investigated the expression of the MERS-CoV receptor, DPP4, in lung tissues of smokers without airflow limitation and COPD patients in comparison to never-smokers. As previously reported, DPP4 is mainly detected in the alveolar epithelial cells of the lungs, the main target of MERS-CoV infection [13, 15]. Among the dendritic cells, we found that DPP4 mRNA is mainly expressed in pDCs; confirming in vitro data showing that among the antigen presenting cells, pDCs produce large amounts of type I and III interferon upon contact with MERS-CoV [14]. Most importantly, we provide evidence that DPP4 is upregulated in the lungs, both at mRNA and protein level, not only in COPD patients but also in that of smokers. This indicates that these individuals may be more susceptible to MERS-CoV, supporting both smoking and COPD as risk factors for MERS-CoV infection [18]. These results are in line with a recent study describing a higher DPP4 expression in lungs of 4 COPD patients compared to 16 control subjects of different ages [13].

In this study, we did not find any evidence of DPP4 upregulation in the bronchial and bronchiolar epithelium in the lungs of smokers and COPD patients, suggesting that DPP4 upregulation in pulmonary epithelia is restricted to the alveolar epithelial cells. Previous studies have shown that DPP4 is limitedly expressed in the bronchial and bronchiolar epithelium, and even absent at the apical surface of the nasal respiratory and olfactory epithelium of humans [13, 15]. Future studies are needed to assess whether DPP4 upregulation is specific for the alveolar epithelial cells or also occurs in the upper respiratory tract epithelium. Additionally, the importance of alveolar macrophages in the pathogenesis of MERS-CoV needs further research as these cells also express DPP4 and patrol the alveoli while being in close contact with the alveolar epithelial cells.

It is currently unclear how DPP4 is upregulated in the lungs of smokers and COPD patients. Several cytokines have been reported to upregulate DPP4 in vitro. TGF-β2, for instance, could upregulate DPP4 protein expression and enzymatic activity in primary human endothelial cells [27], whereas interleukin (IL) 13 has been reported to increase DPP4 mRNA expression in human primary bronchial epithelial cells [28]. On the other hand, in COPD pathogenesis, several cytokines—such as IL-6, IL-8, and the TGF-β

superfamily—have been described to play important roles [29, 30]. Further studies are needed to identify cytokines that could both upregulate DPP4 in the lung and influence COPD pathogenesis.

We also showed that DPP4 mRNA and protein expression were inversely correlated with lung function and diffusing capacity parameters. These data suggest a possible role of DPP4 in COPD pathogenesis. DPP4 is an exopeptidase responsible for cleaving chemokines and this alters the biological function. Moreover, DPP4 is able to activate T cells and induce production of pro-inflammatory cytokines, which later could affect the development of COPD [20, 31–33]. Furthermore, DPP4 is also capable of influencing migration of immune cells by activating or deactivating chemokines in an inflammatory or tumor environment [10, 11]. Interestingly, soluble DPP4 in the serum of COPD patients has been reported to be significantly lower compared to that of non-COPD controls [34, 35]. It remains possible that in COPD patients, DPP4 concentration is low in the serum and high in the lungs to facilitate migration of certain immune cells into or out of the lungs.

Our study has several strengths; first, we included a large number of patients which have been thoroughly characterized. Second, to eliminate the possible interference of the presence of malignancy in our patients, we also included lung tissue derived from explant lungs of end-stage COPD patients, devoid of malignancy. A possible limitation of our study might be the sex imbalance in the groups with, respectively, a male predominance in the COPD groups and a female predominance in the never-smokers. However, it should be noted that linear regression analyses indicated that sex does not significantly contribute to the differences in expression of membrane-bound DPP4. Our data are in line with recent analyses of soluble DPP4 in serum in patients with COPD versus non-COPD controls indicating that there is no relationship between sex and DPP4 levels [35]. It is also important to acknowledge that there are other factors related to COPD that could contribute to the increased MERS-CoV susceptibility independent of DPP4. For instance, COPD patients are mostly in advanced age and more prone to many other pulmonary infections during hospitalization [36]. Besides that, COPD is associated with systemic inflammation, which might also cause insufficient host immune response against pathogens [20, 37].

In conclusion, because smoking is the most common etiology of COPD [19], our data highlight the association between chronic exposure to cigarette smoking and DPP4 upregulation in the lungs, as well as partially explain the increased susceptibility of smokers and COPD patients to MERS-CoV infection [18]. It is imperative to try replicating this observation in an animal model in order to further dissect the molecular pathway of DPP4 upregulation in the lungs.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. W. J. reports grants from Boehringer Ingelheim, Astra Zeneca, Novartis, Chiesi, GSK, outside the submitted work; G. F. J. reports grants from AstraZeneca, grants from Boehringer Ingelheim, grants from Chiesi, grants from GlaxoSmithKline, grants and personal fees from Novartis, personal fees from Teva, outside the submitted work. All other authors have no reported disclosures. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Author notes

B. L. H. and G. G. B. contributed equally to this manuscript.