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509

Cystic fibrosis transmembrane conductance regulator positively regulates angiotensin-converting enzyme 2 expression and SARS-CoV-2 viral entry into airway epithelial cells: Implications for patients with cystic fibrosis

V. Bezzerri[1,2], V. Gentili[3], C. Boni[1,2], E. Baldisseri[1,2], A. Finotti[4], C. Papi[4], A. Tamanini[5], D. Olioso[6], A. Polimeni[1], S. Leo[7], M. Borgatti[4], S. Volpi[1], P. Pinton[7], G. Cabrini[4], R. Gambari[4], F. Blasi[8,9], G. Lippi[6], A. Rimessi[7], R. Rizzo[3], M. Cipolli[1]. *[1]Cystic Fibrosis Center of Verona, Azienda Ospedaliera Universitaria Integrata, Verona, Italy; [2]Section of Molecular Pathology, Department of Pathology and Diagnostics, University Hospital of Verona, Verona, Italy; [3]Chemical and Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy; [4]Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [5]Section of Molecular Pathology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy; [6]Section of Clinical Biochemistry, Department of Neurosciences, Biomedicine and Movement, University of Verona, Verona, Italy; [7]Medical Sciences, University of Ferrara, Ferrara, Italy; [8]Respiratory Unit and Adult Cystic Fibrosis Center, Department of Internal Medicine, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; [9]Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy*

Background: Respiratory viral infections (respiratory syncytial and influenza A viruses) have been found to be associated with deterioration of pulmonary function and exacerbations in people with cystic fibrosis (CF) [1]. Conversely, PwCF infected by SARS-CoV-2 are not experiencing worse clinical outcomes than the general population, except for lung-transplanted patients, who are more likely to require oxygen therapy and hospitalization and are six times as likely to die. Several studies have reported that PwCF with SARS-CoV-2 have a lower case fatality rate than the general population [2–4]. We hypothesized that loss of CF transmembrane conductance regulator (CFTR) protein expression and function may provide an advantage against severe COVID-19 outcomes.
Methods: We investigated expression of Angiotensin-converting enzyme 2 (ACE2) and CD13 (*ANpEp*) messenger ribonucleic acid (mRNA) and protein in CF and non-CF bronchial epithelial (CFBE) cells, including polarized CFBE41o$^-$, 16HBE14o$^-$, Calu-3, and air-liquid interface (ALI)-differentiated primary nasal and bronchial epithelia. Cell susceptibility to SARS-CoV-2 infection was assayed by infecting single cells with a multiplicity of infection of 0.1 for 2 hours. Twenty-four, 48, and 72 hours after infection, RNA was isolated from cells and supernatants, and SARS-CoV-2 titration was obtained using a TaqMan assay by quantitative polymerase chain reaction and digital polymerase chain reaction. IL-6 levels were quantified by enzyme-linked immunosorbent assay in supernatants from primary airway epithelial cells stimulated with 1 mg/mL of SARS-CoV-2 spike protein for 12 hours.
Results: Expression of SARS-CoV-2 receptor ACE2 is significantly downregulated in CF cells in terms of mRNA and protein. We consistently observed that expression of the aminopeptidase CD13, which has been found co-expressed with ACE2 in several tissues and acts as a co-receptor for other human coronaviruses, is equally downregulated. We reported that the F508del-mutated CFTR channel promotes mislocalization of ACE2, which is almost completely retained into the endoplasmic reticulum, similar to unfolded CFTR, suggesting an association between these two proteins. Most importantly, lower ACE2 expression in CF cells is associated with less SARS-CoV-2 viral entry and replication. Eventually, spike-induced IL-6 levels were significantly lower in ALI-differentiated primary airway epithelia obtained from people with CF, consistent with the lower ACE2 expression.
Conclusions: This study clarifies why SARS-CoV-2 infection does not promote particularly severe outcomes in people with CF, unless they have received lung transplantation. Our results also showcase CFTR as a regulator of ACE2 and SARS-CoV-2 viral entry and replication.
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510

Prevalence and clinical impact of respiratory viral infections in the Standardized Treatment of Pulmonary Exacerbations 2 Study

C. Thornton[1], L. Kalikin[1], D. Sanders[2], S. McClellan[3], W. LeBar[3], D. VanDevanter[4], S. Heltshe[5,6], L. Caverly[1], J. LiPuma[1]. *[1]Pediatrics, University of Michigan Medical School, Ann Arbor, MI; [2]Pediatrics, Indiana University, Indianapolis, IN; [3]Pathology, University of Michigan Medical School, Ann Arbor, MI; [4]School of Medicine, Case Western Reserve University, Cleveland, OH; [5]Pediatrics, University of Washington, Seattle, WA; [6]Cystic Fibrosis Foundation Therapeutics Development Network Coordinating Center, Seattle Children's Research Institute, Seattle, WA*

Background: Respiratory symptoms are hallmarks of pulmonary exacerbations (PEx) and viral infections. Although viral respiratory infection rates are similar in persons with cystic fibrosis (PwCF) and the general population, the association between PEx and viral infection is unclear. The Standardized Treatment of Pulmonary Exacerbations II (STOP2) study was a multicenter, randomized clinical trial in PwCF aged 18 and older to evaluate response to various intravenous antibiotic durations for PEx. Sputum collected during study visits provided an opportunity to evaluate respiratory viral infection prevalence and clinical impact in the context of PEx.
Methods: Sputum, when available, was collected between 3 days before therapy to 1 day after initiation of therapy (Visit 1), day 7 to 10 of therapy (Visit 2), and 2 weeks after treatment completion (Visit 3) and shipped to the University of Michigan. Samples were tested for 17 respiratory viruses using multiplex-polymerase chain reaction (FilmArray Respiratory Panel 2, BioFire Diagnostics, LLC). Differences in lung function (percentate predicted forced expiratory volume in 1 second; FEV_1pp) and symptom (Chronic Respiratory Infection Symptom Score; CRISS) changes between visits were compared as a function of viral detection using analysis of covariance. Demographic variables were assessed using logistic or linear regression.
Results: We analyzed 1,323 samples from 690 STOP2 participants. One or more viruses were detected in 393 samples (30%) from 269 participants (39%). Participants who were virus positive at Visit 1 had significantly greater mean decrease in FEV_1pp from the preceding 6 months (5.8 ± 1.3% vs. 3.6 ± 0.9%, $p = 0.03$), worse CRISS (53.8 ± 2.1 vs. 51.1 ± 1.0, $p = 0.03$), and greater CF transmembrane conductance regulator modulator use (47% vs. 34%, $p < 0.001$). The 68% of virus-positive participants with rhino or enterovirus had fewer PEx in the prior year (53%) than other virus-positive participants (66%) ($p = 0.03$). Adjusted mean FEV_1pp, CRISS, and weight changes from Visit 1 to 3 were similar in virus-negative and -positive participants, except for greater mean FEV_1pp change from Visit 2 to 3 in participants who were virus positive at Visit 1 (0.12 ± 0.9 vs. – 1.4 ± 1.0, $p = 0.04$). Participants co-infected with two or more viruses had greater mean FEV_1pp change from Visit 1 to 3 (10.8 ± 4.1, $p = 0.04$) and from Visit 2 to 3 (1.2 ± 2.3, $p = 0.04$) and greater CRISS improvement from Visit 1 to 2 (– 20.7 ± 5.2, $p = 0.03$) than those with one or no viruses. No significant differences in mean FEV_1pp, CRISS, or weight change across visits were observed between rhino or enterovirus infection and other viruses. Concordance in viral detection across visits was observed in 69% of the 176 participants with samples available for all three visits. Subjects with negative samples at all visits had lower CRISS change from Visit 1 to 2 (-13.3 ± 2.4, $p = 0.02$) and Visit 1 to 3 (-14.1 ± 2.4, $p = 0.04$) than those with