compelling basic science and animal model observations would provide a solid foundation upon which to implement additional trials of "anti-coagulants" in IPF. In conclusion, we have answered the question of the utility of warfarin in progressive IPF, but many questions remain.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Imre Noth, M.D.
University of Chicago
Chicago, Illinois

Mitchell Olman, M.D.
Cleveland Clinic
Cleveland, Ohio

On behalf of the IPFnet investigators

References

1. Noth I, Anstrom K, Calvert SB, de Andrade J, Flaherty KR, Glazer C, Kaner RJ, Olman MA. A placebo-controlled randomized trial of warfarin in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2012;186:88–95.
2. Kubo H, Nakayama K, Yanai M, Suzuki T, Yamaya M, Watanabe M, Sasaki H. Anticoagulant therapy for idiopathic pulmonary fibrosis. *Chest* 2005;128:1475–1482.
3. Gunther A, Mosavi P, Ruppert C, Heinemann S, Temmesfeld B, Velcovsky HG, Morr H, Grimminger F, Walmrath D, Seeger W. Enhanced tissue factor pathway activity and fibrin turnover in the alveolar compartment of patients with interstitial lung disease. *Thromb Haemost* 2000;83:853–886.
4. Wygrecka M, Kwapiszewska G, Jablonska E, von Gerlach S, Henneke I, Zakrzewicz D, Guenther A, Preissner KT, Markart P. Role of protease-activated receptor-2 in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;183:1703–1714.
5. Scotton CJ, Krupiczojc MA, Königshoff M, Mercer PF, Lee YC, Kaminski N, Morser J, Post JM, Maher TM, Nicholson AG, *et al*. Increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury. *J Clin Invest* 2009;119:2550–2563.
6. Finigan JH, Dudek SM, Singleton PA, Chiang ET, Jacobson JR, Camp SM, Ye SQ, Garcia JG. Activated protein C mediates novel lung endothelial barrier enhancement: role of sphingosine 1-phosphate receptor transactivation. *J Biol Chem* 2005;280:17286–17293.
7. Fryer A, Huang YC, Rao G, Jacoby D, Mancilla E, Whorton R, Piantadosi CA, Kennedy T, Hoidal J. Selective O-desulfation produces nonanticoagulant heparin that retains pharmacological activity in the lung 1,2. *J Pharmacol Exp Ther* 1997;282:208–219.
8. Koenig A, Norgard-Sumnicht K, Linhardt R, Varki A. Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins: implications for the use of unfractionated and low molecular weight heparins as therapeutic agents. *J Clin Invest* 1998;101:877–889.
9. Garg HG, Thompson BT, Hales CA. Structural determinants of antiproliferative activity of heparin on pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L779–L789.

Copyright © 2012 by the American Thoracic Society

A Young Hispanic with c.1646G>A Mutation Exhibits Severe Cystic Fibrosis Lung Disease: Is Ivacaftor an Option for Therapy?

To the Editor:

Cystic fibrosis (CF) is a lethal autosomal recessive inherited disease caused by the loss or dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride (Cl^-) channel activity resulting from mutations (1, 2). More than 1,600 mutations, which can be broadly grouped into six classes, have been identified in the CFTR gene (3). The incidence of CF and the frequency of specific mutations have been found to vary among ethnic populations (4, 5).

We presented a clinical case of a young Hispanic patient with CF heterozygous for c.1521_1523delCTT (p.Phe508del or F508del)/c.1646G>A (p.Ser549Asn or S549N) mutations (please note that the legacy names for the mutations will be used in this letter). This individual was diagnosed with CF at 5 months of age after multiple hospitalizations due to dehydration, failure to thrive, and emesis. She also had a prolonged and persistent cough thought secondary to a diagnosis of pertussis at 4 months of age. At diagnosis, she was also noted to have a hypochloremic, hypokalemic metabolic alkalosis. She was below the fifth percentile on all aspects of her growth curve. Sweat chloride levels were abnormal with values of 87.1 mmol/L (right arm) and 78.2 mmol/L (left arm). She was empirically given pancreatic replacement enzymes and fat soluble vitamin replacement. By the age of 3 years, she was found to be colonized by two strains of nonmucoid *Pseudomonas aeruginosa* as well as a mucoid *Pseudomonas* that was her predominant isolate by 4 years of age. Over the past 2 years, she has had three additional hospitalizations for pulmonary exacerbations (April 2010, April 2011, and October 2011). Pulmonary function tests (PFTs) have continued to worsen with FEV_1 values of 50 to 60% predicted (the most recent FEV_1 prior to hospitalization in October 2011 was 51% predicted) and $FEF_{25–75}$ values in the low end of 20 to 40% predicted (the most recent value was 20% predicted). She has had increasing pulmonary symptoms and has developed worsening sinusitis. Chest computed tomography scan completed in October 2011 showed areas of extensive bronchiectasis and fibrotic change, predominantly affecting the upper lobes. Areas of disease were observed in the lower lobes as well (Figure 1A and Figure E1 in the online supplement). There are numerous areas of "tree-in-bud" opacities present essentially involving all lobes of both lungs (Figure 1A).

F508del is the most common CFTR mutation, with more than 90% of patients with CF carrying it on at least one allele. F508del-CFTR protein is insufficiently folded and is trapped in the endoplasmic reticulum and targeted for degradation (1). Like c.1652G>A (p.Gly551Asp or G551D), S549N mutation occurs in the signature sequence (LSGGQ) of the first nucleotide-binding domain on the CFTR protein (Figure E2A). S549N mutation was first reported by Curtis and colleagues in a Pakistani individual who was homozygous for S549N and demonstrated severe malnutrition, growth retardation, and advanced pulmonary disease (6). S549N mutation was found to be prevalent among Hispanic, African American, and Asian populations (4).

We characterized S549N-CFTR at the protein level to understand its molecular characteristics and the associated severe disease phenotype and, more importantly, to explore using mutation-specific therapy for medical interventions. S549N-CFTR was expressed as a mature form of CFTR (band C) with levels comparable to wild-type CFTR (Figure 1B). Surface labeling assay and fluorescence microscopy data showed that S549N-CFTR was expressed at the plasma membrane of cells (Figures E3A and E3B). However, S549N-CFTR lacks Cl^- channel function (Figures E2B and E2C). These molecular characteristics correlate well with the severe CF phenotype we observed and suggest that, like G551D, S549N-CFTR is probably a regulation mutant.

Author Contributions: The manuscript was written by S.Y. and W.Z. supervised by A.P.N. and D.C.S. The project was designed and supervised by A.P.N. S.Y. conducted site-directed mutagenesis and surface labeling, F.A.I.K. performed iodide efflux assays, and H.P. performed immunoprecipitation and Western blotting experiments. A.R. conducted I_{sc} measurements. K.A. performed immunofluorescence microscopy studies. C.A.D., S.S., and D.C.S. did the clinical studies. J.C.K. assisted in site-directed mutagenesis. All authors discussed the results and commented on the manuscript.

Supported in part by U.S. National Institutes of Health funding (DK074996 and DK080834 to A.P.N.) and U.S. Cystic Fibrosis Foundation funding to D.C.S.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

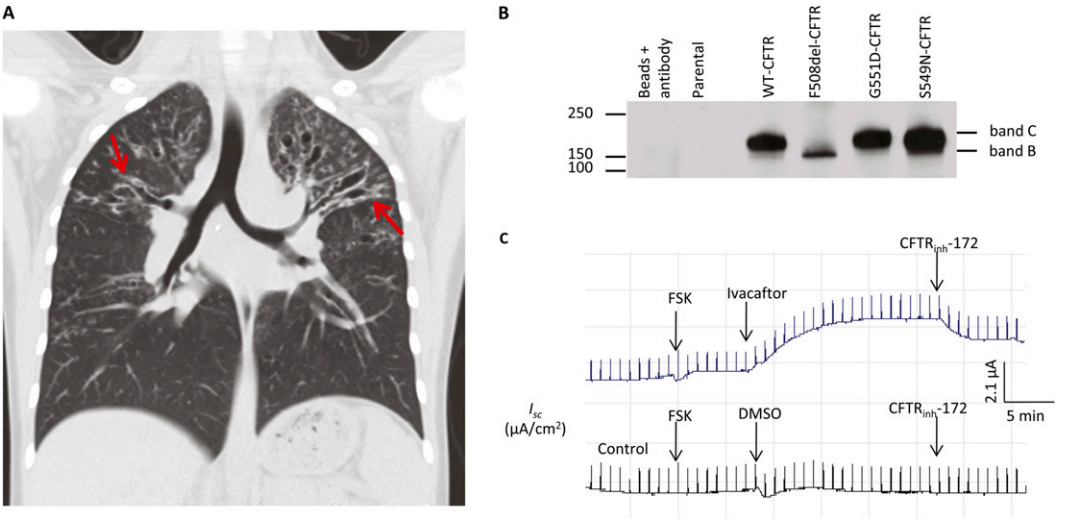

Figure 1. S549-CFTR is a regulation mutant and associates with a severe cystic fibrosis (CF) lung disease in a young Hispanic patient with CF. (*A*) Chest computed tomography (coronal view) shows extensive bilateral upper lobe bronchiectasis (*arrows*, left > right) with relatively less involvement of the lower lung fields. (*B*) S549N-CFTR has normal protein expression levels. HEK-293 cells were transiently transfected with pcDNA3 containing WT, S549N, G551D, or F508del cDNA, lysed 48 hours after transfection, immunoprecipitated with α-24-1 monoclonal antibody and Western blotted for CFTR by using α-NBD-1-R polyclonal antibody. (*C*) Ivacaftor (100 μM) restored Cl^- channel function of S549N-CFTR expressed in $CFBEo^-$ cells. The cells were grown on Costar Transwell permeable supports until they reached resistance of more than 1,400 Ω and then mounted in an Ussing chamber. FSK (10 μM) was used to activate CFTR channel function and $CFTR_{inh}$-172 was used to verify the observed signal were CFTR mediated. DMSO (the solvent for Ivacaftor) was used as a negative control. *See* Reference 12 for technical details. DMSO = dimethyl sulfoxide; WT = wild-type; FSK = forskolin.

Ivacaftor (VX-770) is a CFTR potentiator that can restore the defective gating of G551D and several other regulation mutants (7–9) and has been approved by U.S. Food and Drug Administration for treating patients with CF aged 6 and older with G551D mutation (10). We expressed S549N-CFTR in polarized human cystic fibrosis bronchial epithelial cells ($CFBEo^-$) and tested the effect of ivacaftor on CFTR channel function. Our results showed that ivacaftor restored the defective channel function of S549N-CFTR (Figure 1C), suggesting that ivacaftor has potential clinical benefit for patients with CF with S549N mutation. Our biochemical findings are consistent with data from a recent study in which ivacaftor was found to potentiate the channel open probability and total Cl^- transport of S549N-CFTR expressed in Fischer rat thyroid cells (9). To further confirm S549N is a regulation mutant and can be rescued by CFTR potentiators, we also tested the effect of another known CFTR potentiator, P1, on the channel function of S549N-CFTR expressed in $CFBEo^-$ cells or human embryonic kidney (HEK-293) cells and found that P1 exerted a potentiating effect similar to that of ivacaftor (Figures E4 and E5).

Because this subject in our study is heterozygous for F508del/S549N mutations, she would be an ideal candidate for the ongoing combinational trials using VX-809 (11) and ivacaftor and, most likely, will benefit from these trials.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Sunitha Yarlagadda, B.Sc., M.Sc.
Weiqiang Zhang, Ph.D.
Himabindu Penmatsa, Ph.D.
Aixia Ren, Ph.D.
Kavisha Arora, B.S.
Anjaparavanda P. Naren, Ph.D.
The University of Tennessee Health Science Center
Memphis, Tennessee

Fatima Anmol I. Khan, B.S.
The University of Tennessee Health Science Center
Memphis, Tennessee
and
Christian Brothers University
Memphis, Tennessee

Catherine A. Donnellan, M.D.
Saumini Srinivasan, M.D.
Dennis C. Stokes, M.D., M.P.H.
The University of Tennessee Cystic Fibrosis Care and Research Center at LeBonheur Children's Hospital
Memphis, Tennessee

John C. Kappes, Ph.D.
The University of Alabama at Birmingham
Birmingham, Alabama

References

1. Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, O'Riordan CR, Smith AE. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* 1990;63:827–834.
2. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993;73:1251–1254.
3. Penmatsa H, Frederick CA, Nekkalapu S, Conoley VG, Zhang W, Li C, Kappes J, Stokes DC, Naren AP. Clinical and molecular characterization of S1118F-CFTR. *Pediatr Pulmonol* 2009;44:1003–1009.
4. Rohlfs EM, Zhou Z, Heim RA, Nagan N, Rosenblum LS, Flynn K, Scholl T, Akmaev VR, Sirko-Osadsa DA, Allitto BA, *et al*. Cystic fibrosis carrier testing in an ethnically diverse US population. *Clin Chem* 2011;57:841–848.
5. Sugarman EA, Rohlfs EM, Silverman LM, Allitto BA. CFTR mutation distribution among Hispanic US and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. *Genet Med* 2004;6:392–399.
6. Curtis A, Richardson RJ, Boohene J, Jackson A, Nelson R, Bhattacharya SS. Absence of cystic fibrosis mutations in a large Asian population sample and occurrence of a homozygous S549N mutation in an inbred Pakistani family. *J Med Genet* 1993;30:164–166.
7. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, Turnbull A, Singh A, Joubran J, Hazlewood A, *et al*. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc Natl Acad Sci U S A* 2009;106:18825–18830.
8. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Dřevínek P, Griese M, McKone EF, Wainwright CE, Konstan MW, *et al*.; VX08–770–102 Study Group. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011;365:1663–1672.
9. Yu H, Burton B, Huang CJ, Worley J, Cao D, Johnson JP Jr, Urrutia A, Joubran J, Seepersaud S, Sussky K, *et al*. Ivacaftor potentiation of multiple CFTR channels with gating mutations. *J Cyst Fibros* 2012;11:237–245.

10. Cystic Fibrosis Foundation. FDA Approves Kalydeco (VX-770)—first drug that targets the underlying cause of cystic fibrosis [accessed 2012 January 31]. Available from: http://www.cff.org/aboutCFFoundation/NewsEvents/1-31-FDA-Approves-Kalydeco.cfm
11. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, Decker CJ, Miller M, McCartney J, Olson ER, *et al*. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc Natl Acad Sci U S A* 2011;108:18843–18848.
12. Li C, Krishnamurthy PC, Penmatsa H, Marrs KL, Wang XQ, Zaccolo M, Jalink K, Li M, Nelson DJ, Schuetz JD, Naren AP. Spatiotemporal coupling of cAMP transporter to CFTR chloride channel function in the gut epithelia. *Cell* 2007;13:940–951.

Copyright © 2012 by the American Thoracic Society

Rod-like Bacteria and Recurrent Venous Thromboembolism

To the Editor:

A 52-year-old male developed abdominal pain and diarrhea (five to six times daily) accompanied by fever (38.5°C) for 3 days. He was admitted with the preliminary diagnosis of acute enteritis. One week after hospitalization, abdominal pain alleviated, but chest tightness and swelling of the right lower limb occurred. Chest computed tomography and ultrasonography showed acute pulmonary embolism and deep vein thrombosis (DVT) of the right lower limb. After anticoagulation therapy with low-molecular-weight heparin, he was discharged after symptoms improved. However, DVT recurred several times. Abdominal ultrasonography showed suspected thrombosis in the left portal vein, and oral warfarin was administered. Six months after venous thromboembolism (VTE), this patient was again admitted to our hospital due to DVT in the left lower limb. In addition to routine examination and immune examination, electron microscopy was performed to observe the morphology of blood cells, revealing that white blood cell count increased (11.5×10^9/L; range: 4–10×10^9/L); CD_3^+T: 50.6% (range: 60–85%). Levels of CD_4, CD_8, CD_{16+56}, and CD_{19} were normal. Expressions of IL-1, IL-2, IL-2 receptor, and tumor necrosis factor-α were normal. IL-4 was 108.9 pg/ml (normal: <39.2), IL-6 was 36.85 pg/ml (normal: <31.2), and IL-8 was 101.41 pg/ml (normal: <48.7).

Electron microscopy showed apoptosis and nucleus disappearance of phagocytes. Rod bacteria–like microorganisms were identified in the phagocytes (Figures 1A–1D). CD_3^+T cells were reduced, but the levels of IL-4, IL-6, and IL-8 increased, suggesting Th1/Th2 imbalance.

Infection causes venous thrombosis in multiple organs (1). Patients with acute pulmonary embolism and chronic thromboembolic pulmonary hypertension had compromised function of CD_3^+T cells and CD_8^+T cells, respectively. The increase in CD_4^+T/CD_8^+T ratio suggested that the occurrence of VTE was related to the immune dysfunction (2, 3). The patient had sometimes withdrawn the intake of warfarin from the first onset of VTE. This may also be a reason of VTE recurrence, and the compromised immune function is an internal factor of VTE recurrence. There is internal correlation between immune disorder and coagulation dysfunction. Patients with inflammatory bowel disorders, Behçet's disease, HIV infection, and other infections exhibit an increased risk of VTE (4). Smeeth and colleagues (5) reported that infectious diseases in a community setting are linked to a transient increase in the risk of VTE, so infection may be a triggering factor in VTE recurrence.

Author disclosures are available with the text of this letter at www.atsjournals.org.

Lemin Wang, M.D., Ph.D.
Xiaoyu Zhang, M.D.
Qianglin Duan, M.D.

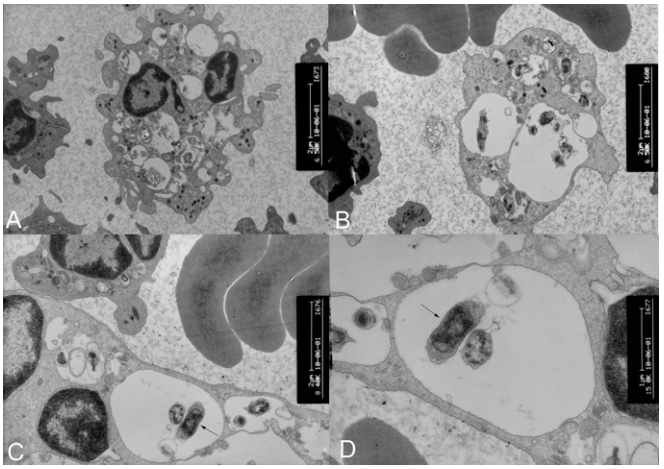

Figure 1. Findings of the neutrophil damage and rod-shaped bacteria-like microorganisms under electron microscope. (*A*) Apoptotic phagocytes. (*B*) The nucleus of phagocytes disappeared. (*C*) Rod-shaped bacteria–like microorganisms were identified in the cytoplasmic cavity of phagocytes (*arrow*). (*D*) Rod-shaped bacteria–like microorganisms at a higher magnification (*arrow*).

Wei Lv, M.D.
Zhu Gong, M.D.
Yuan Xie, M.D.
Aibin Liang, M.D., Ph.D.
Yenan Wang, M.D.
Tongji Hospital
Shanghai, China
and
Medicine School of Tongji University
Shanghai, China

References

1. Xiang-Hua Y, Le-Min W, Ai-Bin L, Zhu G, Riquan L, Xu-You Z, Wei-Wei R, Ye-Nan W. Severe acute respiratory syndrome and venous thromboembolism in multiple organs. *Am J Respir Crit Care Med* 2010;182:436–437.
2. Wang L, Song H, Gong Z, Duan Q, Liang A. Acute pulmonary embolism and dysfunction of CD3+ CD8+ T cell immunity. *Am J Respir Crit Care Med* 2011;184:1315.
3. Haoming S, Lemin W, Zhu G, Aibin L, Yuan X, Wei L, Jinfa J, Wenjun X, Yuqin S. T cell-mediated immune deficiency or compromise in patients with CTEPH. *Am J Respir Crit Care Med* 2011;183:417–418.
4. Prandoni P. Acquired risk factors for venous thromboembolism in medical patients. Hematology/the Education Program of the American Society of Hematology American Society of Hematology Education Program 2005:458–461.
5. Smeeth L, Cook C, Thomas S, Hall AJ, Hubbard R, Vallance P. Risk of deep vein thrombosis and pulmonary embolism after acute infection in a community setting. *Lancet* 2006;367:1075–1079.

Copyright © 2012 by the American Thoracic Society

Proposed Alternative Scoring for SAQLI Domain D

To the Editor:

The Sleep Apnea Quality of Life Index (SAQLI) is a disease-specific quality-of-life (QOL) measure that was first developed and evaluated over 10 years ago (1, 2) and has been widely used since that time. One advantage of the SAQLI over other disease-specific QOL measures is that it allows for patients to select up to five "important" symptoms. This provides a tailored