The Journal of Molecular Diagnostics, Vol. 18, No. 1, January 2016

ELSEVIER

the Journal of Molecular Diagnostics
jmd.amjpathol.org

The Spectrum of *CFTR* Variants in Nonwhite Cystic Fibrosis Patients

Implications for Molecular Diagnostic Testing

Iris Schrijver,*† Lynn Pique,* Steve Graham,‡ Michelle Pearl,§ Athena Cherry,*† and Martin Kharrazi¶

From the Departments of Pathology and Pediatrics,† Stanford University Medical Center, Stanford; the Genetic Disease Screening Program,‡ and the Environmental Health Investigations Branch,¶ California Department of Public Health, Richmond; and the Sequoia Foundation,§ La Jolla, California*

Accepted for publication
July 7, 2015.

Address correspondence to Iris Schrijver, M.D., Department of Pathology, Stanford University Medical Center, L235, 300 Pasteur Dr., Stanford, CA 94305. E-mail: ischrijver@stanfordhealthcare.org.

Despite the implementation of cystic fibrosis (CF) newborn screening programs across the United States, the identification of *CFTR* gene variants in nonwhite populations compared with whites remains suboptimal. Our objective was to establish the spectrum of *CFTR* variants and their frequencies in CF patients in the United States with African, Native American, Asian, East Indian, or Middle Eastern backgrounds. By using direct DNA sequencing, we identified two *CFTR* variants in 89 of 140 affected nonwhite individuals with uncharacterized genotypes. Seven variants were novel. Multiplex ligation-dependent probe amplification detected 14 rearrangements in the remaining 51 patients, 6 of which were novel. Deletions and duplications accounted for 17% of unidentified alleles. A cross-sectional analysis of genotyping data from the CF Foundation Patient Registry was performed, comparing 3496 nonwhite patients with 22,206 white CF patients. Patients of Hispanic, black, or Asian ancestry were less likely to have two identified *CFTR* variants ($P < 0.0001$ for Hispanics and blacks, $P = 0.003$ for Asians), and more likely to carry no mutations on the commonly used 23 mutation carrier screening panel ($P < 0.0001$). We analyzed the mutations recorded for each ancestry and summarized the most frequent ones. This research could facilitate equity in mutation detection between white and nonwhite or mixed-ethnicity CF patients, enabling an earlier diagnosis improving their quality of life. *(J Mol Diagn 2016, 18: 39—50; http://dx.doi.org/10.1016/j.jmoldx.2015.07.005)*

Cystic fibrosis (CF; Online Mendelian Inheritance in Man no. 219700, *http://www.ncbi.nlm.nih.gov/omim*) is one of the most frequent autosomal-recessive conditions. CF has an overall birth prevalence of 1:3500 individuals in the United States.[1] It is most common among non-Hispanic whites (approximately 1:2500) and Ashkenazi Jews (approximately 1:2270) and, consequently, these two populations have been studied the most extensively.[2–4] Among nonwhite populations, CF is less frequent. In the United States, CF occurs in approximately 1:15,000 blacks, 1:35,000 individuals of Asian descent, and 1:10,900 Native Americans.[4,5] CF affects the exocrine epithelial cells of multiple tissues and organs, including the respiratory tract, the pancreas, the intestine, the male genital tract, the hepatobiliary system, and the sweat glands.[6] Morbidity and mortality in CF are attributed most commonly to pulmonary disease, characterized by chronic lung infections and airway inflammation. Other common clinical manifestations are failure to thrive, pancreatic insufficiency, meconium ileus, and infertility resulting from a congenital bilateral absence of the vas deferens.

CF is caused by mutations in the 27-exon *CFTR* gene (Online Mendelian Inheritance in Man no. *602421, *http://www.ncbi.nlm.nih.gov/omim*) that encodes the cystic fibrosis transmembrane conductance regulator (CFTR), a 1480—amino acid protein that forms chloride ion channels in the apical epithelial

Supported by clinical research grants from Cystic Fibrosis Foundation Therapeutics, Inc. (SCHRIJ11A0-NOA47100), and by the CF Foundation, which provided access to data in the CF Foundation Registry.

http://dx.doi.org/10.1016/j.jmoldx.2015.07.005

cell membrane.[7–9] When CFTR production is disrupted, the resulting abnormal electrolyte transport contributes to CF pathogenesis. Defective ion transport in the respiratory tract, in particular, leads to depleted airway surface liquid and increased mucosal obstruction.[10,11] Currently, there are more than 2000 described *CFTR* sequence variants (*http://www.genet.sickkids.on.ca/cftr/StatisticsPage.html*; last accessed June 19, 2015) and these are distributed throughout the gene, but to date only a subset have been firmly established as pathogenic by empiric analyses.[12] The majority of *CFTR* variants are point mutations or other small sequence changes, however, up to 2% of CF alleles likely are gene rearrangements, including large deletions, insertions, and duplications.[13] The most common *CFTR* mutation, p.Phe508del (delF508 by legacy nomenclature), accounts for approximately 66% of identified mutant alleles worldwide.[14] The spectrum and frequency of individual *CFTR* variants, however, vary relative to specific ethnic groups and geographic locations.[4,14,15] For instance, the c.3120+1G>A variant, although uncommon in non-Hispanic whites, is the second most frequent CF allele among black individuals, occurring at a frequency of 10% to 12%.[16,17] Ignoring this single, overall relatively rare allele in, for example, a newborn CF program in a state with black constituents would lower the detection rate for this population and could result in delayed diagnoses.

A clinical diagnosis of CF usually is based on two criteria: the presence of at least one distinctive clinical feature and laboratory evidence of CFTR dysfunction, typically an increased sweat Cl^- concentration.[18] A diagnosis can be made much more rapidly by incorporation of molecular testing and the identification of two *CFTR* mutations.[1] Thus, the diagnosis increasingly is expedited by molecular analysis that can be applied to both symptomatic and presymptomatic patients. Symptomatic identification of CF patients (excluding newborns with meconium ileus or a pre-existing family history) on average delays the diagnosis until 14.5 months of age, resulting in postponed treatment and significant compromises to clinical status.[19,20]

To date, knowledge of the spectrum of *CFTR* variants in nonwhite patients has remained limited. As a result, CF has been diagnosed at a later age among several nonwhite groups compared with whites[19–22] and there likely remains an inequitable identification of CF variants, despite the implementation of newborn screening in all states. Nonwhite patients may be diagnosed more frequently based on symptoms, rather than through a newborn screening algorithm or molecular diagnostic testing because the testing panels in use do not sufficiently include the variants that are prevalent in nonwhite populations. To determine which *CFTR* alleles are prevalent among nonwhite CF patients, we recruited and comprehensively genotyped 140 CF probands of African, Asian, Native American, East Indian, or Middle Eastern ancestry whose molecular etiology had not been fully characterized. Combining our results with genotype data from the CF Foundation Patient Registry, we identified the individual CF variants present in affected nonwhites and estimated their frequencies. Finally, we assessed the proportion of nonwhite patients who would not be identified by commonly used mutation analyses.

Materials and Methods

Study Subjects

Eligible participants included nonwhite CF patients with 0 or 1 identified *CFTR* variants(s) (including patients without genotype testing information) who were enrolled in the CF Foundation Patient Registry via 161 CF Centers in the United States. We excluded variants known to be benign but did not aim to predict or assign pathogenicity to the identified sequence changes. Rather, our focus was to characterize the spectrum of *CFTR* sequence variants. For the purposes of this study, nonwhite patients were those who self-identified as black, Asian, Native American, East Indian, or Middle Eastern; white and Hispanic CF patients were not eligible for the study. CF patients reporting more than one race/ethnicity were not excluded from participation unless they were of mixed white and Hispanic descent. Patient recruitment began in 2009 and, based on non-identifiable data from the CF Foundation Patient Registry and our criteria, 528 living nonwhite CF patients were eligible for participation. Patients were recruited through the CF Centers providing care to these 528 patients. The study coordinator used mail, e-mail, and telephone calls to Center Directors to invite eligible patients identified by Center staff to obtain free *CFTR* sequencing at the clinical Stanford Molecular Pathology laboratory. Over the 4-year study period, 140 patients were enrolled. Results from clinical testing were reported back to the Center and patient. Center staff were expected and reminded by study staff to add testing results into the CF Foundation Patient Registry for later analysis.

Genomic DNA Amplification and CFTR Sequencing

Whole blood was collected via venipuncture at the CF Center and sent to the clinical diagnostic Stanford Molecular Pathology laboratory, where DNA extraction, amplification, and *CFTR* sequencing were performed. Genomic DNA was isolated from peripheral blood using standard procedures. All 27 exons of the *CFTR* gene (*http://www.ncbi.nlm.nih.gov/genbank*, GenBank accession number NG_016465.1) and noncoding regions in which mutations are known to exist [5′ untranslated region, at least 20 bp on each side of an exon, and intervening sequence (IVS)12 and 22 (IVS11 and 19 by legacy numbering)] were PCR-amplified using primer pairs from flanking intronic sequences as originally described[7,23,24] or as modified where needed[25] (and unpublished data). Amplified products were purified using either the Qiaquick PCR Purification Kit or the Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA) according to the