concentrations of the recombinant human S100B.


DOI: 10.1373/clinchem.2003.027367

Multicenter Characterization and Validation of the Intron-8 Poly(T) Tract (IVS8-T) Status in 25 Coriell Cell Repository Cystic Fibrosis Reference Cell Lines for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene Mutation Assays, Silby Sebastian, Silvia G. Spitzen, Leonard E. Grosso, Jean Amos, Frederick V. Schaefer, Elaine Lyon, Daynna J. Wolff, Atieh Hajianpour, Annette K. Taylor, Alison Millson, and Timothy T. Stenzel (1 Department of Pathology, Molecular Diagnostics Laboratory, Duke University Medical Center, Durham, NC; 2 Molecular Genetics Laboratory of SUNY at Stony Brook, Stony Brook, NY; 3 Department of Pathology, St. Louis University School of Medicine, St. Louis, MO; 4 Specialty Laboratories Inc., Santa Monica, CA; 5 Chapman Institute of Medical Genetics, Tulsa, OK; 6 ARUP Laboratories, University of Utah, Salt Lake City, UT; 7 Department of Pathology and Laboratory Medicine, Medical University

I thank C. Acklin for technical assistance, Dr. A. Rowlerseon for critical reading, and D. Arevalo for assistance with the preparation of the manuscript.

References


DOI: 10.1373/clinchem.2003.027367

Multicenter Characterization and Validation of the Intron-8 Poly(T) Tract (IVS8-T) Status in 25 Coriell Cell Repository Cystic Fibrosis Reference Cell Lines for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene Mutation Assays, Silby Sebastian, Silvia G. Spitzen, Leonard E. Grosso, Jean Amos, Frederick V. Schaefer, Elaine Lyon, Daynna J. Wolff, Atieh Hajianpour, Annette K. Taylor, Alison Millson, and Timothy T. Stenzel (1 Department of Pathology, Molecular Diagnostics Laboratory, Duke University Medical Center, Durham, NC; 2 Molecular Genetics Laboratory of SUNY at Stony Brook, Stony Brook, NY; 3 Department of Pathology, St. Louis University School of Medicine, St. Louis, MO; 4 Specialty Laboratories Inc., Santa Monica, CA; 5 Chapman Institute of Medical Genetics, Tulsa, OK; 6 ARUP Laboratories, University of Utah, Salt Lake City, UT; 7 Department of Pathology and Laboratory Medicine, Medical University
Cystic fibrosis (CF) is the most common life-limiting recessive genetic disorder in Caucasians, with a carrier frequency of \(~1\) in 25 and incidence of \(~1\) in 2500–3300 live births (1). CF is caused by mutations affecting the transmembrane conductance regulator (CFTR) gene localized on the long arm of chromosome 7 (7q31.2). CFTR contains 27 exons and encodes a protein of 1480 amino acids that functions as a cAMP-regulated chloride channel in the apical membrane of epithelial cells (2, 3). Mutations in the CFTR gene lead to dysfunction of the lungs, sweat glands, testes, ovaries, intestines, and pancreas. More than 1000 mutations in this gene have been identified to date (4). The clinical manifestations of the disease are variable, ranging from severe pulmonary disease with pancreatic insufficiency to mild pulmonary disease and pancreatic sufficiency (1). Moreover, mutations in the CFTR gene have also been found in patients who have normal lung function but show other clinical signs, such as congenital bilateral absence of the vas deferens (CBAVD), nasal polyposis, bronchiectasis, and bronchopulmonary allergic aspergillosis (5, 6).

Some of the variability in the CF phenotype has been attributed to the influence of the 5T allele at a polymorphic poly(T) tract in intron 8 (IVS8-T) of the CFTR gene. Genotype–phenotype correlations have shown that there is a strong association of the 5T allele with male infertility caused by congenital CBAVD and with other nonsymptomatic forms of CF, such as bronchiectasis and chronic idiopathic pancreatitis (5–7). At the IVS8-T locus, which functions as a splice acceptor site, three variants designated 5T, 7T, and 9T have been identified. The 5T variant is a poor splice acceptor site and gives rise to skipping of exon 9 in a high percentage of CFTR mRNA transcripts (7–11). CFTR mRNA missing exon 9 does not produce a functional protein. A CFTR gene with the 5T allele produces only 5% of the normal concentration of normal mRNA, and when coupled with a CF mutation, this can have a clinical effect (9).

There are three main molecular scenarios that are clinically relevant: (a) When 5T is present with a severe CF mutation on the opposite chromosome (in trans), individuals may be asymptomatic, may have mild symptoms of CF, or if male, may have CBAVD (5, 6). (b) The 5T allele modifies the penetrance of the mild CF mutation R117H. When R117H is on the same allele (in cis) with 5T and another CF mutation is present on the other chromosome, the outcome is usually mild CF with pancreatic insufficiency, although some cases of classic pancreatic-insufficient CF have been seen (12, 13). (c) In males, R117H in trans with 5T (without the presence of another CF mutation) is associated with CBAVD (5, 13).

The 7T variant also plays a clinical role. When R117H is in cis with 7T and another CF mutation is present on the other chromosome (in males), CBAVD may result with or without late onset of mild lung disease (6, 12). The American College of Medical Genetics has recommended reflex testing for the 5T/7T/9T variant when the R117H mutation is found (1, 13, 14). If 5T is present, further testing of parents or offspring is recommended to determine whether the 5T is in cis or trans with R117H. This increased diagnostic relevance of CFTR IVS8-T status in fully evaluating genotype–phenotype correlation in CF and CBAVD prompted us to devise methods to determine poly(T) tract status in CFTR.

The present study was undertaken to characterize IVS8-T status in the cell lines of the CFTR mutation panel (Order No. MUTCF) provided by the Coriell Cell Repository. The panel contains 21 of the 25 alleles recommended by the American College of Medical Genetics for routine diagnostic and carrier testing and is widely used for procedure validation and positive control samples in CF testing. However, IVS8-T status of CFTR in this mutation panel has not been reported. Additionally, we validated these results with eight other molecular diagnostic laboratories that routinely conduct CF testing to establish the potential utility of these CFTR cell lines as test controls in determining CFTR IVS8-T tract variant status. We determined IVS8-T allele status in all 21 cell lines included in the Coriell CFTR mutation panel (MUTC) and in 4 additional Coriell CF cell lines (NA11290, NA13032, NA13033, and NA07464). We tested the cell lines with the INNO-LiPA CFTR 17+Tn (Innogenetics), a line probe assay system based on the reverse hybridization principle (http://www.innogenetics.com/site/diagnostics.html). To verify IVS8-T results for samples NA11275 and NA11280, which showed discrepant test results at one participating laboratory, IVS8-T status was additionally tested by use of the ELUCIGENE™ CF-PolyT ASR system (Orchid Biosciences), which uses the ARMSTM technology (http://www.elucigene.co.uk/pdf/PolyT_UK.pdf). In all cases, instructions provided by the manufacturers were followed with modifications. For the INNO-LiPA system, instead of following the manufacturer’s instructions to process each strip (CFTR16 and CFTR170) in separate troughs, we routinely processed both strips in the same trough by placing one strip facing down and other facing up. In the case of the INNO-LiPA system, the amount of genomic DNA used in the PCR was in the range of \(~1000–3000\) ng, and for the ELUCIGENE assay, it was 50 ng. To further confirm the T-allele status in samples NA11275 and NA11280, we performed nucleotide sequencing using the Big Dye Terminator v.1.1 Cycle sequencing reagent set (Applied Biosystems).

The data were compared with the IVS8-T allele status found by eight other well-established molecular diagnostic laboratories that routinely conduct CF assays. Laboratories 2, 3, 4, and 6 used LINEAR ARRAY CF Gold 1.0 supplied by Roche Diagnostics Corporation (http://www.roche-applied-science.com/pack-insert/3253660A.pdf); lab-
The results of IVS8-T allele status in 25 DNA samples containing well-characterized CFTR mutations are depicted in Table 1. The results agree in all but two samples, NA11275 and NA11280, for which the results reported by laboratory 4 differed from the results reported by the others. Nucleotide sequencing confirmed the consensus T-allele status (shown in the “Consensus” column).

In conclusion, our study determined and validated the IVS-8 T allele status in all 25 cell lines routinely used as test controls in CF assays. The commercial availability of IVS-8 T allele status in all 25 cell lines routinely used as test controls in CF assays could facilitate interlaboratory standardization and proficiency testing for these clinically relevant CFTR mutations.


DOI: 10.1373/clinchem.2003.028068