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Development of Genomic Reference Materials for Cystic Fibrosis Genetic Testing

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The number of different laboratories that perform genetic testing for cystic fibrosis is increasing. However, there are a limited number of quality control and other reference materials available, none of which cover all of the alleles included in commercially available reagents or platforms. The alleles in many publicly available cell lines that could serve as reference materials have neither been confirmed nor characterized. The Centers for Disease Control and Prevention-based Genetic Testing Reference Material Coordination Program, in collaboration with members of the genetic testing community as well as Coriell Cell Repositories, have characterized an extended panel of publicly available genomic DNA samples that could serve as reference materials for cystic fibrosis testing. Six cell lines [containing the following mutations: E60X (c.178G>T), 444delA (c.312delA), G178R (c.532G>C), 1812–1G>A (c.1680–1G>A), P574H (c.1721C>A), Y1092X (c.3277C>A), and M1101K (c.3302T>A)] were selected from those existing at Coriell, and seven [containing the following mutations: R75X (c.223C>T), R347H (c.1040G>A), 3876delA (c.3744delA), S549R (c.1646A>C), S549N (c.1647G>A), 3905insT (c.3773_3774insT), and I507V (c.1519A>G)] were created. The alleles in these materials were confirmed by testing in six different volunteer laboratories. These genomic DNA reference materials will be useful for quality assurance, proficiency testing, test development, and research and should help to assure the accuracy of cystic fibrosis genetic testing in the future. The reference materials described in this study are all currently available from Coriell Cell Repositories. *(J Mol Diagn 2009, 11:186–193; DOI: 10.2353/jmoldx.2009.080149)*

Cystic fibrosis (CF) is an autosomal recessive disorder that affects approximately one of every 2500 live births (Caucasian). The *CFTR* gene was cloned in 1989 and to date over 1500 mutations linked to CF have been identified (The Hospital for Sick Children, *http://www.genet.sickkids.on.ca/cftr*, accessed May, 13, 2008). In 2001 the American College of Medical Genetics (ACMG) and the American College of Obstetricians and Gynecologists (ACOG) recommended *CFTR* mutation carrier screening be offered during pregnancy or in anticipation of a pregnancy to couples who were non-Jewish Caucasian or Ashkenazi Jewish.[1] A panel of 25 *CFTR* mutations with an allele frequency ≥0.1% in the general U.S. population was recommended for screening. The recommendation was updated by ACOG in 2005 to offer screening to all couples regardless of race or ethnicity.[2] In 2004, the mutation screening panel was revised to 23 mutations[3] and the ACMG developed a recommended newborn screening panel that included CF.[4] In response to these initiatives both *CFTR* testing volumes and the available commercial platforms for testing have undergone extensive growth.

Suggestions for more standardization and the expansion beyond the recommended CF testing panels have generated much discussion in the genetic testing community.[5] Although inclusion of additional CF alleles does not significantly increase the detection frequency in European Caucasians, many commercial reagents and platforms exceed the recommended ACMG/ACOG 23 mutation panel. Two out of the four Food and Drug Administration-cleared CF assays exceed the recommended screening panel, suggesting that platform manufacturers perceive a competitive edge for screening additional CF mutations.

Reference materials are needed for test development and validation, lot-testing of new reagent batches and for performance evaluation (proficiency testing/external quality assessment) programs. In addition, international, federal, and state regulations and professional guidelines require the use of reference or quality control materials alongside patient samples,[6–11] (European Molecular Genetics Quality

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Network, *http://emqn.org/eqmn/bestpractice.html*, 05/13/2008; Washington State Legislature, *http://apps.leg.wa.gov/WAC/default.aspx?cite_246-338*, 05/13/2008; New York State Clinical Laboratory Evaluation Program, *http://www.wadsworth.org/labcert/clep/clep.html*, 05/13/2008; College of American Pathologists, *http://www.cap.org,* 05/13/2008; American College of Medical Genetics, *http://www.acmg.net/Pages/ACMG_Activities/stds-2002/g.htm*, 06/02/2008). Despite the increase in the number of laboratories performing CF testing and the ever expanding cadre of CF mutation panels, characterized genomic DNA reference materials for mutations outside of the ACMG/ACOG 23 mutation panel are not available for laboratory quality assurance purposes. In the absence of these materials, laboratories, test developers and proficiency test providers must rely on residual patient specimens, which are often difficult to find and not consistently available (General recommendations for quality assurance programs for laboratory molecular genetic tests, *http://wwwn.cdc.gov/dls/genetics/qapt.aspx*, 12/11/2008).[12]

The Centers for Disease Control and Prevention-based Genetic Testing Reference Materials Coordination Program (*http://wwwn.cdc.gov/dls/genetics/rmmaterials/default.aspx*, 05/13/2008), together with the clinical genetics laboratory community initiated a project to obtain and characterize additional publicly available cell lines and genomic DNA reference materials for CF genetic testing. These materials supplement the available characterized genomic DNA reference materials, which cover the recommended mutation screening panel. When we began this study, the National Institute of General Medical Sciences Human Genetic Cell Repository at the Coriell Cell Repositories already had cell lines covering the 23 alleles recommended by ACMG/ACOG, which were characterized previously by DNA sequence analysis (Coriell Cell Repositories, Camden, NJ). We selected additional alleles commonly included in commercial CF reagents that are not included in the 23 ACMG/ACOG alleles but are present in relatively high frequency in ethnic populations,[3] alleles that were already available from Coriell but that had not been independently confirmed and alleles representing polymorphisms that could interfere with detection of F508del (c.1521_1523delCTT), which is one of the 23 alleles recommended by ACMG, and a mutation that could interfere with the detection of S549N (c.1647G>A), an allele common in some ethnic populations. Six cell lines were selected from those existing at Coriell and nine new cell lines were created specifically for this project. DNA samples were prepared from these cell lines by Coriell. The CF alleles in these materials were confirmed by testing in six volunteer laboratories using a variety of assay platforms, including DNA sequence analysis. We also documented the previously unpublished characterization of DNA from a number of other Coriell CF cell lines. These genomic DNA samples are publicly available from Coriell and can be used for quality assurance, assay development, and validation, as well as for proficiency testing. The availability of these reference materials will support accurate clinical CF testing.

Materials and Methods

Cell Line Creation and DNA Preparation

After patient testing, residual whole blood containing CF mutations was sent to the Coriell Cell Repositories, under an existing institutional review board research protocol at the collection site, for Epstein-Barr virus transformation of B-lymphocytes as previously described.[13,14] All samples were placed into culture and expanded to yield approximately 2×10^8 total viable cells. The culture medium was antibiotic-free to increase the likelihood that contamination would be readily detected. The cell suspension was dispersed in 40 1-ml vials so that each contained 5×10^6 viable cells. Cultures were cryopreserved in heat-sealed borosilicate glass ampoules and stored in liquid nitrogen (liquid phase). Successful cultures were free from bacterial, fungal, and mycoplasmal contamination and were viable after cryopreservation in liquid nitrogen, as evidenced by a doubling of the cell number within four days of recovery. Approximately 2 mg of DNA was prepared from each of the selected cell lines by Coriell Cell Repositories using Gentra/Qiagen Autopure per manufacturer's instructions (Valencia, CA) or previously described methods.[15]

Laboratory Selection

A total of six clinical genetic laboratories that offer CF testing volunteered to participate in the study. Laboratories were solicited based on their current CF assay methods, so that each of the DNA samples was tested by all of the commonly used platforms. The assay method used, alleles included in this study, and whether or not they can be detected by the assay are shown in Table 1. The assays used in this study detect the 23 alleles recommended by ACMG/ACOG as well as additional CF alleles. All of the laboratories are located in the United States and have CLIA certification or are accredited by the College of American Pathologists (CAP).

CF Assays Used in the Characterization Study

The assays and platforms used in this study are as follows:

Luminex Tag-It CFTR 40 + 4 Platform

The Tag-It CFTR 40 + 4 Mutation Detection Kit (Luminex Molecular Diagnostics, Austin TX) simultaneously screens for 40 mutations and four variants. Briefly, genomic DNA was amplified and alleles were discriminated using allele-specific primer extension and hybridization to a universal microsphere array. Genotypes were detected on a Luminex 100 IS System and called using the proprietary TM Data Analysis Software as previously described.[16]

Asuragen Signature 2.0 CF Expand Platform

The Asuragen Signature 2.0 CF Expand reagents (Asuragen Inc., Austin, TX) simultaneously screens for 44 mutations and four variants. Genomic DNA was amplified in

Table 1. Specificity of Detection Methods

Study alleles		Methods*					
cDNA sequence change†	Common name	Luminex Tag-It	Asuragen Signature	Laboratory developed test (Luminex)	Third Wave InPlex	Abbott/Celera OLA	Sequencing
c.3302T>A	M1101K	yes‡	no	yes	no	no	yes
c.3277C>A	Y1092X	yes	no	yes	yes	no	yes
c.1721C>A	P574H	no	no	yes	no	no	yes
c.532G>C	G178R	yes	no	yes	no	no	yes
c.312delA	444delA	yes	no	yes	no	no	yes
c.1680-1G>A (AJ574983.1: g.92G>A)	1812-1G>A	yes	yes	yes	no	no	yes
c.1040G>A	R347H	yes	yes	yes	yes	yes	yes
c.3744delA	3876delA	yes	yes	yes	yes	yes	yes
c.1647G>A	S549N	yes	yes	yes	yes	yes	yes
c.3773_3774insT	3905insT	yes	no	yes	yes	yes	yes
c.1646A>C	S549R	yes	no	yes	yes	yes	yes
c.1516A>G	I506V	yes	no	yes	yes	yes	yes
c.1519A>G	I507V	yes	no	yes	no	yes	yes
c.223C>T	R75X	no	no	yes	no	no	yes
c.178G>T	E60X	no	no	yes	yes	no	yes

*Methods used: Luminex Tag-It CFTR 40 + 4 Mutation Detection Kit (FDA-cleared; IVD); Asuragen Signature 2.0 CF Expand [laboratory developed test (LDT)]; Laboratory developed assay; Third Wave InPlex (LDT); Abbott/Celera OLA V3.0 (LDT); sequencing (LDT).

†HGVS nomenclature *http://www.hgvs.org/mutnomen/* (accessed December 9, 2008). All cDNA sequence change nomenclature is based on the GenBank cDNA reference sequence NM_000492.3.

‡Yes indicates allele is detected by the method, no indicates allele is not detected by method.

a single multiplex PCR reaction using fluorescently labeled primers. The PCR products were denatured and hybridized to allele-specific oligonucleotides coupled to Luminex x-Map Beads (Luminex, Austin, TX). Genotypes were detected on a Luminex 100 IS System and called using the proprietary Signature Script software.[17]

Laboratory Developed Test on Luminex Platform

This proprietary method for the detection of 97 CF mutations includes the amplification of genomic DNA followed by allelic discrimination using allele-specific primer extension and hybridization to a universal microsphere panel. Genotypes were detected on the Luminex 100 xMAP system and assigned using TM Data Analysis Software. Alleles on multiplexed microspheres were resolved by bidirectional sequencing.

Third Wave CFTR InPlex Platform

The CFTR InPlex (Third Wave Technologies, Madison, WI) assay detects 38 clinically relevant mutations and six variants. Briefly, genomic DNA was amplified using an abbreviated PCR and the products (15 μL) were added to the CFTR InPlex cards containing fluorescent resonance energy transfer cassettes to detect the mutations after isothermal signal amplification. Results were read on a plate reader and genotypes were called by the Invader® Data Analysis Worksheet software.[18]

Abbott/Celera Oligonucleotide Ligation Assay Platform

The Abbott/Celera oligonucleotide ligation assay reagents (Alameda, CA) detect 31 clinically relevant mutations and six variants. Briefly, genomic DNA was amplified by PCR and the products were subsequently used in the oligonucleotide ligation assay reaction. The oligonucleotide ligation assay products were detected on the Applied Biosystems, Inc. (ABI) 3100 Genetic Analyzer and interpreted using the ABI CF Genotyper software (Foster City, CA).[19]

DNA Sequence Analysis

DNA sequence analysis was performed in two different laboratories. One laboratory used Big Dye terminator chemistry with detection on an ABI3100 and/or ABI 3730 Genetic Analyzer. Primers were designed to amplify the exons containing the mutations under standard amplification conditions. Sequencing was performed in the forward and reverse directions and compared with a normal in-house control. The second laboratory performed DNA sequence analysis as previously described.[20]

Protocol

Each of the six testing laboratories received one 50-μg aliquot of DNA from each of the 15 CF cell lines tested. The expected CF allele(s) in each of the samples were not revealed to the laboratories except to those who were performing DNA sequence analysis (laboratory staff was told which exons to sequence, but blinded to the specific mutation). The laboratories assayed each DNA sample using their standard assay methods (Table 1). One laboratory performed testing of the DNA from the cell lines using two different CF assays as it was validating a new platform at the time of the study. These results were sent to the study coordinators (LVK and VMP), who examined the data for quality and discrepancies.

Table 2. Characterized Cell Lines

Coriell #	cDNA sequence change name* Allele 1	cDNA sequence change name* Allele 2	Common name Allele 1	Common name Allele 2	Methods† (# labs)	Population frequency of mutated allele(s)‡	Refs.§
GM07441¶	c.2988 + 1G>T‖ (AJ575003.1:g.305G>T)	c.489 + 1G>T‖ (AJ574942.1: g.240G>T)	3120+G>A‖	621 + 1G>T‖	A(4), C(1), E(1), F(1), G(2), H(1)	0.86%/1.30%**	O,P
GM13591¶	c.1521_1523delCTT‖	c.350G>A‖	F508del‖	R117H‖	A(5), C(1), E(1), F(1), G(2), H(1)	66.31%/0.54%**	O,P,Q
GM18799¶	c.1521_1523delCTT‖	c.2052delA‖	F508del‖	2184delA‖	A(1)	66.31%/0.15%**	O,Q
GM18800¶	c.1521_1523delCTT‖	c.1766 + 1G>A‖ (AJ574983.1:g.179G>A)	F508del‖	1898 + 1G>A‖	A(1)	66.31%/0.13%**	O,Q
GM11277¶	c.1519_1521delATC‖		del1507‖		A(1)	0.90%**	O,Q
GM01531¶	c.1521_1523delCTT‖	c.1521_1523delCTT‖	F508del‖	F508del‖	A(1)	66.31%/66.31%**	O,Q
GM11496¶	c.1624G>T‖	c.1624G>T‖	G542X‖	G542X‖	A(1)	2.64%/2.64%**	O,Q
GM07552¶	c.1521_1523delCTT‖	c.1657C>T‖	F508del‖	R553X‖	A(1)	66.31%/1.21%**	O,Q
GM08338¶	c.1652G>A‖		G551D‖		A(1)	1.93%**	O,Q
GM11859¶	c.2657 + 5G>A‖ (AJ574995.1:g.216G>A)	c.2657 + 5G>A‖ (AJ574995.1:g.216G>A)	2789 + 5G>A‖	2789 + 5G>A‖	A(1)	0.38%/0.38%**	O,Q
GM11723¶	c.3846G>A‖		W1282X‖		A(1)	2.20%**	O,Q
GM11860¶	c.3718-2477C>T‖ (AY848832.1:g.40725C>T)	c.3718-2477C>T‖ (AY848832.1:g.40725C>T)	3849 + 10kbC>T‖	3849 + 10kbC>T‖	A(1)	0.85%/0.85%**	O,Q
GM11280¶	c.579 + 1G>T‖ (AJ574943.1: g.261G>T)	c.489 + 1G>T‖ (AJ574942.1: g.240G>T)	711 + 1G>T‖	621 + 1G>T‖	A(1)	0.35%/1.30%**	O,Q
GM11282¶	c.254G>A‖	c.489 + 1G>T‖ (AJ574942.1: g.240G>T)	G85E‖	621 + 1G>T‖	A(1)	0.26%/1.30%**	O,Q
GM12585¶	c.3484C>T‖		R1162X‖		A(1)	0.3%**	O,Q
GM12444¶	c.1585-1G>A‖ (AJ574980.1: g.116G>A)		1717-1G>A‖		A(1)	0.44%**	O,Q
GM11472¶	c.4046G>A	c.3909C>G‖	G1349D	N1303K‖	A(1)	no data/1.27%**	O,Q
GM12785¶	c.1652G>A‖	c.4040G>C‖	G551D‖	R347P‖	A(1)	1.93/0.36%**	O,Q
GM12960¶	c.1000C>T‖		R334W‖		A(1)	0.37%**	O,Q
GM11275¶	c.3437delC‖	c.1521_1523delCTT‖	3659delC‖	F508del‖	A(1)	0.28%/66.31%**	O,Q
GM11281¶	c.1521_1523delCTT‖	c.489 + 1G>T‖ (AJ574942.1: g.240G>T)	F508del‖	621 + 1G>T‖	A(1)	66.31%1.30%**	O,Q
GM11283¶	c.1521_1523delCTT‖	c.1364C>A‖	F508del‖	A455E‖	A(1)	66.31%/0.26%**	O,Q
GM11284¶	c.1521_1523delCTT‖	c.1679G>C‖	F508del‖	R560T‖	A(1)	66.31%/0.30%**	O,Q
GM07461	c.1657C>T‖		R553X‖		A(1)	1.21%**	Q
GM08345	c.1521_1523delCTT‖	c.1521_1523delCTT‖	F508del‖	F508del‖	A(1)	66.31%/66.31%**	Q
GM11278	c.1521_1523delCTT‖	c.1477C>T	F508del‖	Q493X	A(1)	66.31%/0.17%**	Q
GM11287	c.1521_1523delCTT‖	c.1721C>A	F508del‖	P574H	A(1)	66.31%¶/very low††	Q
GM12961	c.1558G>T		V520F		A(1)	0.09%**	Q
GM13033	c.1523T>G		F508C		A(1)	no data	Q
GM13423	c.254G>A‖	c.3454G>C	G85E‖	D1152H	A(1)	0.26%/0.03%**	Q
CD00003	c.948delT		1078delT		B(1), C(1), L(1)	0.03%**	P
CD00004	c.1766 + 1G>A‖ (AJ574983.1:g.179G>A)		1898 + 1G>A‖		B(1), C(1), L(1)	0.13%**	P
CD00005	c.1766 + 1G>A‖ (AJ574983.1:g.179G>A)		1898 + 1G>A‖		B(1), C(1), L(1)	0.13%**	P
CD00006	c.1766 + 1G>A‖ (AJ574983.1:g.179G>A)		1898 + 1G>A‖		B(1), C(1), L(1)	0.13%**	P
CD00007	c.1766 + 1G>A‖ (AJ574983.1:g.179G>A)		1898 + 1G>A‖		B(1), C(1), L(1)	0.13%**	P
CD00008	c.2052delA+		2184delA‖		B(1), C(1), L(1)	0.15%**	P
CD00009	c.262delTT		394delTT		B(1), C(1), L(1)	0.09%††	P
CD00010	c.443T>C		I148T		B(1), C(1), L(1)	0.08%**	P
CD00012	c.443T>C		I148T		B(1), C(1), L(1)	0.08%**	P
CD00013	c.3703A>C		S1235R		A(6), D(1), J(1), K(2)	1.6%‡‡	P

*All cDNA sequence change HCVS nomenclature is based on the GenBank cDNA reference sequence NM_000492.3.

†Methods used: A, sequencing; B, reverse hybridization; C, oligonucleotide ligation assay (Abbott/Celera); D, mass spectrometry; E, allele specific amplification assay with gel electrophoresis; F, Invader Assay (Third Wave Technologies); G, restriction fragment length polymorphism; H, restriction fragment length polymorphism + sequencing; I, heteroduplex analysis; J, restriction fragment length polymorhpism + heteroduplex analysis + sequencing; K, eSensor chip (Motorola Life Sciences) + sequencing; L, 97 Mutation allele-specific assay (proprietary LDT); M, heteroduplex analysis + sequencing; N, restriction fragment length polymorphism + heteroduplex analysis + oligonucleotide ligation assay.

‡CTFR mutation frequency among clinically diagnosed cystic fibrosis individuals in a pan-ethnic U.S. population.

§References for characterization: O, Coriell ACMG23 Panel (MUTCF); P, Reference #23, Q, DNA sequencing Michele Caggana, NYSDOH.

¶Cell line included in Coriell MUTCF Panel.

‖Allele included in the ACMG 23 Panel.

**See reference # 3.

††See reference # 21.

‡‡See reference # 22.

Additional Alleles Characterized

In addition, several DNA variants present in the publicly available cell lines, which were not specifically targeted in this study, were confirmed by DNA sequence analysis performed previously at the Wadsworth Center, New York State Dept. of Health. These cell lines and the alleles that they contain are indicated in Table 2.

Results

Twenty-three cell lines (Table 2, cell lines with †) containing the 23 mutations recommended for screening by ACMG/ACOG (Table 2, alleles with *) have been characterized previously and are available as a reference material panel from Coriell (Coriell Cell Repositories, Camden, NJ). For this study, we selected DNA samples containing 15

Table 3. Results of Multi-Laboratory Characterization Studies

Coriell #	cDNA sequence change name*		Common name		Population frequency of allele(s)‡
	Allele 1 (# labs†)	Allele 2 (# labs†)	Allele 1 (# labs†)	Allele 2 (# labs*)	
GM07857	c.3302T>A (3)	c.3302T>A (3)	M1101K (3)	M1101K (3)	0.20%/0.20%§
GM11285	c.1521_1523delCTT (6)	c.3277C>A (4)	F508del (6)	Y1092X (4)	66.31%‡/0.12%¶
GM11287	c.1521_1523delCTT (6)	c.1721C>A (2)	F508del (6)	P574H (2)	66.31%‡/very low§
GM11288	c.1521_1523delCTT (6)	c.532G>C (3)	F508del (6)	G178R (3)	66.31%‡/0.2%§
GM11370	c.312delA (3)	c.1680-1G>A (AJ574983.1:g. 92G>A (4)	444delA (3)	1812-1G>A (4)	0.05§/0.02%¶
GM20737	c.1040G>A (6)	Wt (6)	R347H (6)	wt (6)	0.06%¶
GM20741	c.3744delA (6)	Wt (6)	3876delA (6)	wt (6)	0.03%¶
GM20745	c.1647G>A (6)	Wt (6)	S549N (6)	wt (6)	0.14%¶
GM20836	c.3773_3774insT (5)	Wt (6)	3905insT (5)	wt (6)	0.12%¶
GM20925	c.1646A>C (5)	Wt (6)	S549R (5)	wt (6)	0.12%§
GM20928	c.1521_1523delCTT (6)	wt‖ (6)	F508del (6)	wt‖ (6)	66.31%¶
GM21551	c.1521_1523delCTT (5)	c.1519A>G (4)	F508del (5)	I507V (4)	66.31%¶/no data
GM20924	c.223C>T (2)	Wt (5)	R75X (2)	wt (5)	no data
GM20929	c.1521_1523delCTT (5)	wt‖ (5)	F508del (5)	wt‖ (5)	66.31%¶
GM07732	c.1521_1523delCTT (3)	c.178G>T (3)	F508del (3)	E60X (3)	66.31%/0.12%¶

*All cDNA sequence change HGVS nomenclature is based on the GenBank cDNA reference sequence NM_00492.3.
†Number of laboratories that successfully detected the mutation.
‡CTFR mutation frequency among clinically diagnosed cystic fibrosis individuals in a pan-ethnic U.S. population.
§See Reference #21.
¶See Reference #3.
‖Cell line was tested for I506V mutation; however, the mutation was not detected and all laboratories reported as not detected (wt) instead.

alleles that are not part of the 23 recommended by ACMG/ACOG to characterize based on their inclusion in a number of commercially available CF reagents. Seven of the selected alleles [E60X (c.178G>T), R347H (c.1040G>A), S549N (c.1647G>A), 1812-1G>A (c.1680-1G>A), Y1092X (c.3277C>A), 3876delA (c.3744delA), and 3905insT (c.3773_3774insT)] have also been suggested for inclusion in carrier screening panels based on their frequency in minority populations.[3] Other alleles selected for study were already available from Coriell [R75X (c.223C>T), 444delA (c.312delA), G178R (c.532G>C), P574H (c.1721C>A), M1101K (c.3302T>A)], or are mutations that would interfere with the detection of F508del (c.1521_1523delCTT) [I506V (c.1516A>G), I507V (c.1519A>G)] or S549N [S549R (c.1646A>C)].

The fifteen experimental cell lines containing the 16 additional alleles were chosen for study based on the submitter's description of the CF mutation present. Six pre-existing Epstein-Barr virus-transformed lymphoblast cell lines were selected from the National Institute of General Medical Sciences Repository at the Coriell Cell Repositories (GM07857, GM11285, GM11287, GM11288 GM07732, and GM11370; Table 3). Nine new CF cell lines described in Table 3 (GM20737, GM20741, GM20745, GM20836, GM20925, GM20928, GM21551, GM20924, and GM20929) were generated for this study.

Since the purpose of this project was to develop characterized reference materials that will be useful for many possible applications, we wanted to ensure that the characterization of these materials was as comprehensive and included as many assay platforms as possible. We compared the alleles detected in all commercially available CF reagents/platforms and selected those with the ability to detect the alleles included in our study. Some of the commercially available reagents/platforms (eg, from Osmetech and from Nanogen) were not used in this study because they detect primarily the ACMG/ACOG 23 panel, and not the additional alleles included in this study. We also included DNA sequence analysis and a laboratory developed assay based on the Luminex platform (LDT, Table 1) because of the ability to detect additional CF mutations. Clinical genetics laboratories performing CF testing using the various reagents/platforms were recruited to participate in this study. The assays used by the participating laboratories and the alleles detected by each are indicated in Table 1.

The results of this study are summarized in Table 3. The expected genotype of each DNA sample was confirmed by all assay platforms designed to detect the alleles. The genotype of each DNA sample, and the number of laboratories/assays that detected each allele, and the published population frequency of each allele, are shown in Table 3. DNA from GM20928 and GM20929 was expected to carry the I506V (c.1516A>G) allele based on information from the submitter. However, this variant was not present when DNA was tested on all four of the assay platforms capable of detecting it. In addition, DNA sequence analysis did not detect the I506V (c.1516A>G) allele in either cell line. No false-positive or other discordant results were reported among the laboratories.

Additional CF Alleles Characterized

A panel of DNA from 23 cell lines representing the 23 CF alleles recommended by ACMG/ACOG is available from Coriell (Coriell Cell Repositories, Camden, NJ). The mutations in some these DNA samples had been confirmed by analysis in numerous laboratories as part of the project that created them.[23] Alleles in the remaining ACMG/ACOG 23 samples and DNA samples from some additional CF cell lines (not included in the ACMG/ACOG 23) have been characterized by DNA sequence analysis

(reported in this work). These results are summarized in Table 2.

Conclusions

CF carrier screening is widespread with at least 66 laboratories in the United States currently offering some form of CF testing (National Institutes of Health, www.genetests.com, 05/13/2008). A higher estimate of CF laboratories can be made by examining the number of participants in the CAP CF surveys [150 laboratories in 2007 based on CAP Participant Summaries, MGL 2007 (reviewed by VMP)]. Note the number of laboratories performing CF testing in the CAP surveys is different from the number of laboratories listed in GeneTests. GeneTests is a voluntary registry and the CAP participating laboratories may include laboratories outside of the United States and laboratories that do not offer clinical CF testing. In addition, CF testing is offered in many hospital laboratories and, in the public health setting, to 3 to 4 million newborns per year (National Newborn Screening and Genetics Resource Center, *http://genes-r-us.uthscsa.edu/nbsdisorders.htm*, 08/14/2008). Due to a lack of available materials for use in quality assurance, proficiency testing, assay validation, and research, we undertook this study to expand the number of characterized mutations in cell lines that can be used as reference materials for CF genetic testing.

Cell lines containing 15 alleles not included in the original ACMG/ACOG 23 panel [E60X (c.178G>T), R75X (c.223C>T), 444delA (c.312delA), G178R (c.532G>C), R347H (c.1040G>A), I506V (c.1516A>G), I507V (c.1519A>G), S549R (c.1646A>C), S549N (c.1647G>A), 1812-1G>A (c.1680-1G>A), P574H (c.1721C>A), Y1092X (c.3277C>A), M1101K (c.3302T>A), 3876delA (c.3744delA), and 3905insT (c.3773_3774insT)] were selected for study. These alleles were chosen because they were included in several targeted CF platforms currently available on the market, were in pre-existing Coriell cell lines or because of their increased prevalence in some ethnic populations. All platforms capable of detecting these mutations were successful and all mutations were confirmed by DNA sequence analysis [with the exception of I506V (c.1516A>G)]. We found no discordant results among six different laboratories using seven different methods.

In addition to characterization of 14 non-ACMG/ACOG 23 CF alleles, we also present results of DNA sequence analysis that confirm the alleles in DNA from the Coriell MutCF-2 panel that had not been previously characterized, as well as from many other CF cell lines (Table 2). Together with previously published data, all of the ACMG/ACOG 23 alleles, plus many other clinically important CF alleles have now been characterized.

Included in this study were DNA samples from two different cell lines in which the submitter had identified the polymorphism I506V (c.1516A>G). We were unable to confirm the presence of the I506V (c.1516A>G) allele in either sample. Although this polymorphism is not clinically relevant, it is recommended by ACMG/ACOG as a part of reflex to confirm F508del (p.F508del) homozygotes. It is interesting that the DNA samples were purified from two cell lines derived from different patients. There are four possible explanations for this: 1) the submitter incorrectly identified the polymorphism; 2) there was a sample mix-up during the process of procurement; 3) the cell line was contaminated; or 4) the mutation was lost during the transformation process. The profile of the DNA isolated from each culture, using six highly polymorphic microsatellite markers, was identical to that of the original blood used by the diagnostic laboratory in detecting the original mutation and for establishing each cell culture at the Coriell Cell Repositories; therefore, it is unlikely that sample mix-up or cross contamination occurred during cell culturing or DNA isolation. It is possible that the I506V (c.1516A>G) mutation was lost during transformation of these two blood samples or that the initial detections were technical artifacts. Regardless of the explanation for failure to detect the I506V (c.1516A>G) mutation in DNA from these two cultures, the observation together with published reports of high frequencies of cell line cross-contamination[24] and dramatic changes in cell cultures during extended growth in cultures[25] highlight the importance of characterizing DNA obtained from cell lines before its use in research, or as a reference material in assay development, validation and/or proficiency testing. Although cell repositories such as Coriell have extensive procedures to assure that their cell lines are maintained free of microbial contamination and that they are identity matched to the original sample submitted. The genetic information that the repository provides to users is largely dependent on that provided by the submitter, investigators who use the samples or external verifiers (Coriell Cell Repositories, Camden, NJ). The major goal of the GeT-RM program is to develop newly characterized genomic DNA reference materials and to confirm the genotypic information provided by the submitters of publicly available cell lines and genomic DNA materials. That said, it is still necessary for users of any DNA and cell lines purchased from repositories or otherwise obtained to confirm the genotype and characteristics before use.[26] The documentation provided by this and other GeT-RM characterization studies brings to the forefront the availability and use of characterized materials for quality assurance purposes.

CF mutation analysis is used for a variety of clinical indications including, but not limited to, carrier testing for an individual with a family history of CF or based on population risk, diagnosis of an asymptomatic newborn or infant, identification of familial mutations in a diagnosed individual, or prenatal diagnosis for a high-risk fetus. Since the advent of the ACMG/ACOG recommendations in 2001, CF mutation testing has grown in volume, commercially available platforms/reagents, and performing laboratories. Many commercial reagents and platforms exceed the ACMG/ACOG 23 mutation panel. Of the 11 currently commercially available reagents and platforms, over half (6/11) exceed the number of recommended ACMG/ACOG mutations. This includes two out of the four *in vitro* diagnostic devices (Food and Drug Administration-cleared assays).

The ACMG/ACOG CF mutation panel was created to screen healthy individuals to determine carrier status. It was not designed to identify affected individuals. Since the recommendation for CF to be added to newborn screening, testing for additional mutations that may be at an increased frequency in certain ethnic groups may be warranted. The California Genetic Disease Branch and the Public Health Institute have expanded the number of alleles to 38 and New York has expanded to 40 alleles for newborn screening (California Department of Public Health, *http://www.dhs.ca.gov/pcfh/gdb/html/PDE/CFTableCurrent.html*, 05/13/2008; personal communication with M. Caggana, New York State Department of Health). Many of the alleles characterized in this study are included in the California and New York newborn screening programs [CA: R75X (c.223C>T), S549N (c.1647G>A), 1812-1G>A (c.1680-1G>A), 3876delA (c.3744delA), NY: E60X (c.178G>T), R347H (c.1040G>A), S549R (c.1646A>C), S549N (c.1647G>A), Y1092X (c.3277C> A), 3876delA (c.3744delA), and 3905insT (c.3773_3774insT)]. All of the other additional mutations in the expanded CA and NY programs do not have characterized genomic DNA reference materials that are publicly available.

We do not advocate expansion of screening panels beyond those recommended by the ACMG/ACOG and recognize that testing for some of these alleles may result in only a modest increase in the detection of CF carriers in Caucasian populations. However, we characterized additional alleles as described to help laboratories obtain reference materials covering panels on the market to ultimately assure the quality of the testing process.

Several types of reference materials are publicly available for CF genetic testing in addition to the cell lines and genomic DNA described here. Three companies have developed synthetic DNA controls for CF testing; Molecular Controls, Inc.,[27] Maine Molecular Quality Control, Inc. (Food and Drug Administration-cleared) (Maine Molecular Quality Controls, Scarborough, ME) and AcroMetrix (Benicia, CA) manufacture control materials that cover all 23 of the ACMG/ACOG recommended alleles as well as other commonly assayed alleles. Synthetic controls are useful because they contain multiple alleles in a single sample, and thus provide efficient and cost saving quality control materials. Some commercially available synthetic CF controls are designed to control for the DNA extraction step as well as for the analytic portion of the assay. While cell lines and patient DNA are less efficient for quality control use, genomic DNA represents a material that closely resembles a patient sample in terms of DNA complexity and composition.

These genomic DNA reference materials are now characterized and are useful for quality assurance, proficiency testing, test development and research. Overall, their use will help to ensure the accuracy of CF genetic testing. The CF reference materials, as well as other materials developed by GeT-RM, are available from Coriell Cell Repositories. More information on this and other projects is available at the GeT-RM website: *http://wwwn.cdc.gov/dls/genetics/rmmaterials/default.aspx*.

References

1. Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ: Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. Genet Med 2001, 3:149–154
2. Committee on Genetics, American College of Obstetricians and Gynecologists: ACOG committee opinion. Number 325, December 2005. Update on carrier screening for cystic fibrosis. Obstet Gynecol 2005, 106:1465–1468
3. Watson MS, Cutting GR, Desnick RJ, Driscoll DA, Klinger K, Mennuti M, Palomaki GE, Popovich BW, Pratt VM, Rohlfs EM, Strom CM, Richards CS, Witt DR, Grody WW: Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genet Med 2004, 6:387–391
4. American College of Medical Genetics' Newborn Screening Expert Group: toward a uniform screening panel and system. Genet Med 2006, 8 Suppl 1:1S–252S
5. Grody WW, Cutting GR, Watson MS: The cystic fibrosis mutation "arms race": when less is more. Genet Med 2007, 9:739–744
6. Amos J, Feldman GL, Grody WW, Monaghan K, Palomaki GE, Prior TW, Richards CS, Watson MS: Technical Standards and Guidelines for CFTR Mutation Testing. American College of Medical Genetics 2006, pp1–28
7. Association for Molecular Pathology: Recommendations for in-house development and operation of molecular diagnostic tests. Am J Clin Pathol 1999, 111:449–463
8. Chen B, O'Connell CD, Boone DJ, Amos JA, Beck JC, Chan MM, Farkas DH, Lebo RV, Richards CS, Roa BB, Silverman LM, Barton DE, Bejjani BA, Belloni DR, Bernacki SH, Caggana M, Charache P, Dequeker E, Ferreira-Gonzalez A, Friedman KJ, Greene CL, Grody WW, Highsmith WE Jr., Hinkel CS, Kalman LV, Lubin IM, Lyon E, Payne DA, Pratt VM, Rohlfs E, Rundell CA, Schneider E, Willey AM, Williams LO, Willey JC, Winn-Deen ES, Wolff DJ: Developing a sustainable process to provide quality control materials for genetic testing. Genet Med 2005, 7:534–549
9. Code of Federal Regulations. The clinical laboratory improvement amendments (CLIA) 2007, 42 CFR Part 493
10. MM01-A2 Molecular diagnostic methods for genetic diseases-approved guideline. The clinical and laboratory standards institute (CLSI). 2nd ed. Wayne PA, CLSI, 2006
11. Potter NT, Spector EB, Prior TW: Technical standards and guidelines for Huntington disease. Genet Med 2004, 6:61–65
12. Williams LO, Cole EC, Lubin IM, Iglesias NI, Jordan RL, Elliott LE: Quality assurance in human molecular genetics testing-Status and recommendations. Arch Pathol Lab Med 2003, 127:1353–1358
13. Beck JC, Beiswanger CM, John EM, Satraiano, West D: Successful transformation of cryopreserved lymphocytes: a resource for epidemiological studies. Cancer Epidemiol Biomarkers Prev 2001, 10:551–554
14. Bernacki SH, Stankovic AK, Williams LO, Beck JC, Herndon JE, Snow-Bailey K, Prior TW, Matteson KJ, Wasserman LM, Cole EC, Stenzel TT: Establishment of stably EBV-transformed cell lines from residual clinical blood samples for use in performance evaluation and quality assurance in molecular genetic testing. J Mol Diagn 2003, 5:227–230
15. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988, 16:1215
16. Strom CM, Janeszco R, Quan F, Wang S, Buller A, McGinness M, Sun W: Technical validation of a Tm biosciences luminex-based multiplex assay for detecting the American College of Medical Genetics-recommended cystic fibrosis mutation panel. J Mol Diagn 2006, 8:371–375
17. Hadd AG, Laosinchai-Wolf W, Novak CR, Badgett MR, Isgur LA, Goldrick M, WalkerPeach CR: Microsphere bead arrays and sequence validation of 5/7/9T genotypes for multiplex screening of cystic fibrosis polymorphisms. J Mol Diagn 2004, 6:348–355
18. Johnson MA, Yoshitomi MJ, Richards CS: A comparative study of five technologically diverse CFTR testing platforms. J Mol Diagn 2007, 9:401–407
19. Brinson EC, Adriana T, Bloch W, Brown CL, Chang CC, Chen J, Eggerding FA, Grossman PD, Iovannisci DA, Madonik AM, Sherman DG, Tam RW, Winn-Deen ES, Woo SL, Fung S: Introduction to PCR/

OLA/SCS, a multiplex DNA test, and its application to cystic fibrosis. Genet Test 1997, 1:61–68
20. Strom CM, Huang D, Chen C, Buller A, Peng M, Quan F, Redman J, Sun W: Extensive sequencing of the cystic fibrosis transmembrane regulator gene: assay validation and unexpected benefits of developing a comprehensive test. Genet Med 2003, 5:9–14
21. Heim RA, Sugarman EA, Allitto BA: Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med 2001, 3:168–176
22. Monaghan KG, Feldman GL, Barbarotto GM, Manji S, Desai TK, Snow K: Frequency and clinical significance of the S1235R mutation in the cystic fibrosis transmembrane conductance regulator gene: results from a collaborative study. Am J Med Genet 2000, 95:361–365
23. Bernacki SH, Beck JC, Stankovic AK, Williams LO, Amos J, Snow-Bailey K, Farkas DH, Friez MJ, Hantash FM, Matteson KJ, Monaghan KG, Muralidharan K, Pratt VM, Prior TW, Richie KL, Levin BC, Rohlfs EM, Schaefer FV, Shrimpton AE, Spector EB, Stolle CA, Strom CM, Thibodeau SN, Cole EC, Goodman BK, Stenzel TT: Genetically characterized positive control cell lines derived from residual clinical blood samples. Clin Chem 2005, 51:2013–2024
24. Chatterjee R: Cases of mistaken identity. Science 2007, 315:928–931
25. Hughes P, Marshall D, Reid Y: Parkes H. Gelber C: the costs of using unauthenticated, over-passaged cell lines: how much more data do we need? Biotechniques 2007, 43:575–584
26. Amos Wilson J, Zoccoli MA, Jacobson JW, Kalman L, Krunic N, Matthijs G, Pratt VM, Schoonmaker MM, Tezak Z: Verification and validation of multiplex nucleic acid assays; approved guideline. MM17-A 2008, 28(9)
27. Christensen TM, Jama M, Ponek V, Lyon E, Wilson JA, Hoffmann ML, Bejjani BA: Design, development, validation, and use of synthetic nucleic acid controls for diagnostic purposes and application to cystic fibrosis testing. J Mol Diagn 2007, 9:315–319