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Development of a Genomic DNA Reference Material Panel for Myotonic Dystrophy Type 1 (DM1) Genetic Testing

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Myotonic dystrophy type 1 (DM1) is caused by expansion of a CTG triplet repeat in the 3′ untranslated region of the *DMPK* gene that encodes a serine-threonine kinase. Patients with larger repeats tend to have a more severe phenotype. Clinical laboratories require reference and quality control materials for DM1 diagnostic and carrier genetic testing. Well-characterized reference materials are not available. To address this need, the Centers for Disease Control and Prevention—based Genetic Testing Reference Material Coordination Program, in collaboration with members of the genetic testing community, the National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients and Family Members, and the Coriell Cell Repositories, has established and characterized cell lines from patients with DM1 to create a reference material panel. The CTG repeats in genomic DNA samples from 10 DM1 cell lines were characterized in three clinical genetic testing laboratories using PCR and Southern blot analysis. *DMPK* alleles in the samples cover four of five DM1 clinical categories: normal (5 to 34 repeats), mild (50 to 100 repeats), classical (101 to 1000 repeats), and congenital (>1000 repeats). We did not identify or establish Coriell cell lines in the premutation range (35 to 49 repeats). These samples are publicly available for quality control, proficiency testing, test development, and research and should help improve the accuracy of DM1 testing. *(J Mol Diagn 2013, 15: 518—525; http://dx.doi.org/10.1016/j.jmoldx.2013.03.008)*

Myotonic dystrophy type 1 (DM1) (Steinert disease), the most common form of adult muscular dystrophy, is a dominantly inherited, multisystem disorder that typically affects skeletal, smooth, and cardiac muscle, the eyes, the brain, and endocrine function.[1] Although penetrance is approximately 100% by age 50 years, there is variable expressivity, and mild cases may be misdiagnosed or undiagnosed.[2,3]

DM1 results from an unstable CTG triplet expansion in the 3′ untranslated region of the *DMPK* gene (encodes

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a serine-threonine kinase) located on chromosome 19q13.3.[2,4–6] Individuals not affected by DM1 have 5 to 34 CTG triplet repeats in leukocyte DNA. Patients with *DMPK* alleles in leukocyte DNA with 35 to 49 CTG repeats (premutations) do not have symptoms, but their children have an increased risk of inheriting larger CTG repeats and of having symptoms.[2]

DMPK alleles with CTG repeat expansions >49 lead to a wide spectrum of symptoms that characterize the DM1 phenotype. Alleles >49 CTG repeats are unstable and may expand in length during meiosis, causing offspring to inherit CTG repeats that are longer than those in the parent. These children often display anticipation defined as an earlier age at onset of a more severe phenotype than the affected parent.[3] In general, patients with larger CTG repeat expansions in circulating leukocyte DNA tend to have more severe clinical phenotypes.[7–10] However, most patients display somatic tissue mosaicism in skeletal muscle, heart, and brain, which can complicate prediction of the phenotype severity.[10–13] Patients with 50 to 100 repeats are mildly affected, usually with cataracts and/or mild myotonia, which develop later in adulthood. Those with the classic phenotype (Steinert's disease) have 101 to 1000 CTG repeats in leukocyte DNA and have classic DM1 symptoms, including cataracts, grip myotonia, and distal weakness. The age at onset for the classical group is approximately late teens to 30 years. Patients with congenital or childhood myotonic dystrophy typically present between birth and 10 years of age and have more severe manifestations. These patients typically have >1000 CTG repeats in circulating blood cells.

Molecular genetic testing for DM1 relies primarily on measurement of the number of CTG repeats in the *DMPK* gene isolated from circulating leukocyte DNA. PCR can be used to measure repeat length in *DMPK* alleles up to approximately 100 CTG repeats. Southern blot analysis is used to detect larger expansions. There are currently no US Food and Drug Administration–approved or cleared molecular genetic tests for DM1. All clinical testing is currently performed using tests developed in the individual laboratory.

Clinical laboratories use characterized reference materials for a variety of quality assurance purposes, including test development, test validation, quality control, and for alternative assessment. Proficiency testing programs often distribute characterized genomic DNA reference materials to their participants. The use of reference materials is also mandated by regulatory requirements and professional guidelines for clinical laboratories.[14–20] [American College of Medical Genetics: Standards and Guidelines for Clinical Genetics Laboratories, *http://www.acmg.net/AM/Template.cfm?Section=Publications1*; Washington State Department of Health, *http://www.doh.wa.gov/hsqa/fsl/lqa_home.htm*; College of American Pathologists, *http://www.cap.org/apps/cap.portal?_nfpb=true&_pageLabel=accreditation* (paid subscription required); and New York State Clinical Laboratory Evaluation Program, *http://www.wadsworth.org/clep*, all websites last accessed January 11, 2013.] Reference materials should be well characterized, homogeneous, and closely resemble an actual clinical specimen.[20] Ideally, the laboratory should use a set of reference materials that contain the range of mutation types expected for the test that will be performed. This will allow development and evaluation of assays to detect a range of variants. Reference materials containing a range of CTG repeat lengths (from <35 to several thousand repeats) should be used for development and evaluation of DM1 genetic tests.

There are no commercial or other sources of characterized reference materials for molecular genetic testing of DM1. Laboratories that perform this test typically use characterized genomic DNA from cell lines, such as those available from the National Institute of General Medical Sciences (NIGMS) repository at the Coriell Institute for Medical Research (Camden, NJ) or residual patient samples as reference materials. Laboratories and proficiency testing programs have been unable to obtain many of the necessary reference materials because cell lines and patient samples with the complete range of CTG repeat lengths are not readily available.

To address this need, the Centers for Disease Control and Prevention's Genetic Testing Reference Material (GeT-RM) Coordination Program collaborated with members of the genetic testing community, the NIGMS repository, and the NIH-funded National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients and Family Members.[21] This report describes the characterization of new and preexisting cell lines in three clinical genetic testing laboratories and the development of a DM1 reference material panel for research and improved clinical testing.

Materials and Methods

Cell Line Selection

DM1 cell lines with a range of repeat lengths were identified in the NIGMS repository at the Coriell Institute for Medical Research. Myotonic dystrophy repeat lengths that were not represented in the NIGMS repository were also identified, and patients with these repeat lengths were recruited and asked to donate whole blood for lymphoblast cell line development. DNA from 13 preexisting cell lines from the NIGMS repository and 12 newly established lines were selected for further characterization in one laboratory to identify candidate reference materials.

Anonymous Blood Collection from Consenting Patients with Myotonic Dystrophy

New DM1 cell lines were established through collaboration with the National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients and Family Members at the University of Rochester Medical Center (Rochester, NY). A protocol and study materials were prepared and approved by the University of Rochester

Research Subjects Review Board. Patients with CTG repeats in selected size ranges (30 to 49, 50 to 199, and ≥2000) were recruited by the National Registry through mailed requests. Patients and their families who wished to donate blood for cell line creation returned the signed consent form to the University of Rochester. Consented patients were then mailed a blood collection kit containing instructions for the patient, a letter for the medical care provider who would collect the blood, blood collection tubes labeled with the deidentified research code, and preaddressed and prepaid shipping labels for sending the blood tubes to the Coriell Cell Repositories. When the patient visited his or her physician for a regular visit, blood was collected and sent (deidentified) to Coriell using the return shipping label and materials provided. Coriell does not have access to patients' names or other identifying information. When the blood specimens were received, each was assigned a new number and was prepared for cell line establishment. When the cell line was established, the data manager requested the deidentified clinical information from the patient registry using the corresponding research code.

Establishment of New Cell Lines

Cell line creation was performed as previously described.[22] Whole blood samples collected from consenting patients or their families were sent to the Coriell Cell Repositories for Epstein-Barr virus transformation of B lymphocytes, as previously described.[23,24] All the samples were placed in culture and were 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 dispensed into forty 1-mL ampules containing 5×10^6 viable cells each. Cultures were cryopreserved in heat-sealed borosilicate glass ampules and were stored in liquid nitrogen (liquid phase). Successful cultures were free of bacterial, fungal, and mycoplasma contamination and were viable after cryopreservation in liquid nitrogen, as evidenced by doubling of the cell number within 4 days of recovery.

DNA Preparation

DNA was prepared as previously described.[22] Approximately 2 mg of DNA was prepared from cultures of each of the selected cell lines by the Coriell Cell Repositories using the Gentra Autopure system (Qiagen Corp., Valencia, CA) per the manufacturer's instructions or previously described methods.[25]

Testing Laboratories

Three clinical genetics laboratories that offer testing for DM1 volunteered to participate in the study. All three laboratories are located in the United States and are accredited by the College of American Pathologists.

Reference Material Characterization

Each volunteer laboratory received 20 to 30 μg of DNA from each of the 10 selected DM1 cell lines. The expected repeat length was not revealed to the laboratories. The laboratories genotyped each DNA sample using their standard DM1 assay. The results were collected by the study coordinator (L.K.), who examined the data for discrepancies.

DM1 Assays

Laboratory 1

The number of CTG repeats was determined using PCR specific for *DMPK* alleles with less than approximately 100 repeats.[4] PCR was performed using the following primers: 5′-CTTCCCAGGCCTGCAGTTTGCCCATC-3′ and 5′-GAACGGGGCTCGAAGGGTCCTTGTAGC-3′, with the latter primer being modified by the addition of 6-FAM (6-carboxyfluorescein) to detect the product by capillary electrophoresis. Approximately 200 to 250 ng of genomic DNA was amplified in a total reaction volume of 20 μL. The final concentrations of PCR reagents were as follows: 1× GeneAmp PCR buffer II, 1.2 mmol/L $MgCl_2$, 200 μmol/L deoxynucleotide mix, 10 ng/μL of each primer, and 1.6 U of AmpliTaq Gold polymerase (Applied Biosystems/Life Technologies, Grand Island, NY). Thermocycling conditions in an Applied Biosystems 9700 or 2720 thermal cycler (Applied Biosystems/Life Technologies) were as follows: 95°C × 10 minutes for initial denaturation followed by 25 cycles at 95°C × 30 seconds, 62°C × 30 seconds, and 72°C × 1 minute, with an additional 5 minutes at 72°C after the final temperature cycle. One microliter of the amplified product was mixed with 0.5 μL of GeneScan-500 ROX size standard and 10 μL of Hi-Di formamide (Applied Biosystems/Life Technologies). Immediately before loading onto an Applied Biosystems 3130 or 3130 XL genetic analyzer (Applied Biosystems/Life Technologies), the samples were denatured for 5 minutes at 100°C and then were placed on ice.

Southern blot analysis was performed as previously described[26] using the DNA probe p5B1.4 [a gift from Keith Johnson (Charing Cross and Westminster Medical School, London, UK)]. Genomic DNA (5 μg) was separately digested with 50 U each of EcoRI and BamHI (Promega, Madison, WI) and was separated by electrophoresis on a 24-cm-long 1% SeaKem agarose gel (Lonza, Rockland, ME) containing ethidium bromide (a 0.5-μg/mL final concentration) (Sigma-Aldrich, St. Louis, MO) for approximately 18 hours at 40 V.

Expanded CTG repeat alleles were estimated after autoradiography by comparing migration with a 1-kilobase (kb) DNA ladder (Invitrogen/Life Technologies) on the agarose gel used for Southern blot analysis. Electrophoretic migration of the autoradiographic signal from expanded CTG repeats was measured from the top of the Southern blot autoradiograph and was visually compared with a image of

the ethidium bromide–stained agarose gel (before blotting) that contained a fluorescent ruler overlaying the 1-kb DNA ladder. The fluorescent ruler measures migration from the top of the agarose gel. The autoradiographic signal measurement from the top of the Southern blot filter corresponds to the agarose gel migration distance as measured by the ruler on the image. When the autoradiographic signal from expanded repeats was diffuse, a frequent occurrence during repeat expansion, the midpoint of the signal was used. CTG repeats were estimated by correlating the measurement after autoradiography with the same distance on the agarose gel and then comparing this measurement with electrophoretic migration of the known 1-kb DNA ladder fragments to obtain an estimate of the size of the expanded repeat allele.

Laboratory 2

The *DM*1 gene region containing the expanded CTG repeat was PCR amplified with primer pair 5′-AACGGGGCTC-GAAGGGTCCT-3′ (forward) and 5′-GCCGAAAGAAA-GAAATGGTCTGT-3′ (reverse) in a GeneAmp 9700 PCR system (Applied Biosystems, Foster City, CA). The PCRs were performed using 0.5 U of *Taq* polymerase (Fisher Scientific, Waltham, MA) in a total volume of 25 μL containing 50 ng of genomic DNA, 0.8 μmol/L primers, 1 mmol/L $MgCl_2$, and 0.2 mmol/L dNTPs, 0.1 μCi of ^{32}[P] dCTP (PerkinElmer/NEN Life Sciences, Boston, MA), and 5% dimethyl sulfoxide. The amplification was performed with an initial denaturation at 94°C × 5 minutes, followed by 30 cycles of denaturation at 94°C × 30 seconds, annealing at 60°C × 30 seconds, and extension at 72°C × 30 seconds. The final extension was at 72°C for 10 minutes. The PCR products were separated by electrophoresis on a 6% denaturing polyacrylamide gel and were visualized by autoradiography. Allele repeat number was determined by comparison with standard fragments of known size for which repeat number had been previously determined by DNA sequencing analysis.

Southern blot analysis was also performed using 10 μg of DNA digested with 10 U of SacI restriction enzyme (New England Biolabs, Ipswich, MA) incubated overnight at 37°C. DNA was precipitated with 3 mol/L sodium acetate and resuspended in 45 μL of hydration solution (Qiagen Inc.). All digested DNAs were applied to a 14-cm 1% agarose gel with 1× Tris-acetate-EDTA buffer (pH 8.3). Electrophoresis was performed for 3.5 hours at 94 V. The Bioline HyperLadder I DNA ladder with 14 bands ranging from 200 to 10,000 bp (Bioline, Taunton, MA) was used as the size marker. The DNA was transferred to a positively charged Millipore S4056 nylon membrane (Millipore, Billerica, MA) using the Probe Tech transfer system (Oncor Inc., Gaithersburg, MD) according to the manufacturer's directions. The probe pM10M6[27] was radiolabeled with α-^{32}[P]dCTP, incubated overnight with the membrane at 42°C, washed with 2× standard saline citrate (SSC)/0.1% SDS for 30 minutes twice and once at 65°C with 0.5× SSC/0.1% SDS for 30 minutes, and, finally, autoradiographed for 2 to 5 days using Fuji medical X-ray film (Fisher Scientific).

In the first analysis, bands representing expanded alleles were estimated by comparison of migration distances of bands and smears representing expanded alleles with migration distances of bands in the Bioline HyperLadder I DNA ladder run on the same gel. In the second analysis, band size was recalculated from the original autoradiogram. Distance of migration of bands on the ladder was measured from the origin and was plotted on semilog paper. Distance of migration of the midpoint of the band or smear representing the expanded allele in the samples was measured and size of the expansion was calculated for the measured distance using the log scale from the size standards.

Laboratory 3

PCR was performed using approximately 100 ng of genomic DNA amplified in a total volume of 15 μL using the primers 5′-CTTCCCAGGCCTGCAGTTTGCCCATC-3′ (forward, 5′ end labeled with 6-Fam) and 5′-GAACGGGGCTCGAAG-GGTCCTTGTAGC-3′ (reverse). The final concentration of PCR components was 0.333 μmol/L primers, 1× Q-Solution (Qiagen Inc.), 2% dimethyl sulfoxide (Fisher, Houston, TX), dNTP (200 μmol/L dATP, CTP, and dTTP and 50 μmol/L dGTP; Roche Applied Science, Indianapolis, IN), 167 μmol/L 7-deaza dGTP (Roche Applied Science), 1× ABI buffer without $MgCl_2$, 2 mmol/L $MgCl_2$, and 1.25U of AmpliTaq Gold (Applied Biosystems/Life Technologies). The PCR conditions were as follows: 94°C × 10 minutes, (95°C × 1 minute, 69°C × 2 minutes) 10 cycles, (95°C × 1 minute, 65°C × 1 minute, 72°C × 2 minutes) 20 cycles, 4°C hold using a PTC-100 thermal cycler (MJ Research, Waltham, MA). Two aliquots of PCR products were loaded onto an Applied Biosystems 3730XL analyzer (two aliquots of amplified PCR products were used (dilution 1:10 and dilution 1:25) to get clear results for both normal and abnormal alleles). Diluted PCR products (2 μL) were mixed with a master cocktail of 18 μL of Hi-Di formamide and 0.1 μL of GeneScan-500 ROX molecular weight marker (Applied Biosystems/Life Technologies). Samples were denatured at 95°C for 3 to 4 minutes and were placed on ice for 3 minutes. PCR products were separated by electrophoresis on an ABI 3730XL capillary electrophoresis instrument and were analyzed using GeneMapper software version 4.0 (Applied Biosystems/Life Technologies).

For Southern blot analysis, 8 μg of DNA was digested with 40 U of BglI (New England Biolabs) at 37°C overnight, and an additional 10 U of BglI was spiked into the digest the next day for 2 hours to ensure complete digestion of genomic DNA. DNA was separated by electrophoresis on an 11 × 14-cm 0.8% agarose LE gel (Roche Applied Science) for approximately 2 hours at 100 V and then was increased to 140 V for 2 to 3 hours. Two fragment-sizing ladders were used on each gel: a 1-kb ladder used to size fragments $<$10 kb and a lambda bacteriophage HindIII ladder used to size fragments $\geq$10 kb (Promega). DNA was transferred to a charged

BioBond-Plus nylon membrane (Sigma-Aldrich) using alkaline transfer with a TurboBlotter apparatus (Whatman-GE Healthcare, Piscataway, NJ) overnight. The membrane was baked for 2 hours at 80°C before a prehybridization step at 65°C of ≥4 hours. Hybridization was performed overnight at 65°C. Prehybridization/hybridization buffer consists of 7% SDS (Roche Applied Science), 1.5× SSPE (Sigma-Aldrich), 10% w/v polyethylene glycol 8000 (100 g in 1 L) (Sigma-Aldrich), 250 mg of heparin in 1 L (Sigma-Aldrich), and 200 ng/mL of salmon sperm DNA (Roche Applied Science) (salmon sperm is denatured for 10 minutes at 95°C before adding to hybridization buffer).

The DM1 probe was made from a 1090-bp PCR product generated with primers 9581-F (5′-TCCCATGTAA-GACCCCTCTCTTTCC-3′) and 10670-R (5′-ATTCCCGG-CTACAAGGACCCTTCG-3′). The probe was labeled with α-^{32}[P]dCTP using High Prime reaction (Roche Applied Science). The membrane was washed at 67°C for 15 minutes with 1× low-stringency wash (2× SSC, 0.1% SDS) and at 67°C for 30 minutes with 1× high-stringency wash (0.1× SSC, 0.5% SDS) and then was placed in a cassette with a phosphoimaging screen and was developed using a Storm Imager system (Amersham Bioscience, Pittsburgh, PA) after 1 to 3 days of exposure.

Repeat size measurements were performed using ImageQuant TL version 7.0 software (GE Healthcare Biosciences, Pittsburgh, PA). The migration of each band was measured to the midpoint of each band or smear. Repeat size was calculated using the Cubic Spline Curve algorithm provided with the ImageQuant software.

In the first analysis, all bands <10 kb were sized by comparison with the 1-kb ladder. Larger bands were sized using the lambda HindIII ladder. In the second analysis, all bands were sized by comparison with the 1-kb ladder.

Results

The goal of this study was to create a comprehensive panel of publicly available, human cell line–based genomic DNA reference materials for myotonic dystrophy genetic testing. Clinical laboratory directors and representatives of a proficiency testing program were consulted to identify specific reference material needs for DM1 molecular genetic testing. This group suggested that genomic DNA reference materials should be developed that include a range of CTG repeat sizes, representing all five clinical categories: normal (5 to 34 repeats), premutation (35 to 49 repeats), mild DM1 (50 to 100 repeats), classical DM1 (101 to 1000 repeats), and congenital DM1 (>1000 repeats).[2]

When this study was initiated, the NIGMS repository at Coriell had DM1 cell lines with repeat lengths ranging from 300 to 2000 repeats. There were also two cell lines with approximately 50 to 80 repeats. There were no DM1 cell lines with CTG repeat lengths at or near the clinical decision points (35 to 49 CTG repeats). The GeT-RM advisors recommended that additional cell lines with repeats in the smaller range (35 to 200 repeats) and repeats in the very large range (>2000, especially >4000) should be added to complement the NIGMS collection and to create a comprehensive set of DM1 reference materials.

New cell lines with these repeat lengths were created through collaboration with the National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy Patients and Family Members at the University of Rochester Medical Center. Using an institutional review board–approved protocol, blood samples from consenting patients with needed *DMPK* repeat lengths were collected, deidentified, and sent to Coriell to create cell lines. Twenty-four blood samples were received, and 17 new cell lines were established. No cell lines with repeat sizes near the clinical decision points (35 to 49 repeats) were obtained despite repeated efforts. Several attempts were made to create cell lines with repeat sizes in the 100 to 200 range. All six lines made by collection of blood samples from registry patients with sizes expected to be 90 to 180 triplet repeats (GM23378, GM23299, GM23374, GM23300, GM23256, and GM23391) were found to have much larger repeats when the cell line DNA was analyzed (Table 1).

Table 1 Characteristics of Preexisting and Newly Created DM1 Cell Lines

	No. of repeats	
Coriell ID	As per Coriell or Registry	As per laboratory 1
GM06075*	66	55/69 mosaic†
GM03990	50–80	78
GM23265*‡	50–60	75
GM23258‡	70–80	79
GM23378*‡	80–90	145
GM23299‡	90–100	475 mosaic
GM23374‡	130–140	475 band not tight
GM23300‡	150–160	550 mosaic
GM23256‡	160–170	775
GM23391†	170–180	775
GM05164*	340	325
GM03756	<500	450 mosaic
GM03986	<500	550 mosaic
GM03697*	<500	370
GM04567*	700	630
GM23358‡	800–900	800 band not tight
GM05212	900–1000	875
GM03696*	<1000	700
GM04034	<1000	700
GM04648*	1000	950
GM06077	1600	1700
GM05152*	1500	1600
GM03132*	<2000	1950

*Cell lines selected for further analysis.

†Mosaic: more than one expanded band or smear occurred in the PCR or Southern blot analysis. Samples with a single repeat value indicate a single predominant signal.

‡Newly created cell lines from DM1 patient blood donations.

Twenty-three candidate genomic DNA samples (14 preexisting and 9 newly created) were tested in one volunteer laboratory (laboratory 1) to confirm the reported CTG repeat length and to assess their suitability as reference materials. This laboratory used PCR and Southern blot analysis. The results are shown in Table 1. Based on this initial assessment of the repeat size in each sample, DNA from 10 cell lines (NA03696, NA03132, NA05164, NA04648, NA03697, NA06075, NA05152, NA04567, NA23378, and NA23265) that best represented the full range of clinically relevant CTG repeat lengths were selected for further characterization in the other two volunteer laboratories.

The CTG repeat sizes of both *DMPK* alleles in the 10 candidate reference materials, as measured by each laboratory, are shown in Table 2. The three laboratories tested each sample using PCR and Southern blot techniques. PCR is a more accurate method than Southern blot analysis to determine CTG repeat length; however, this method cannot be used reliably for alleles with more than approximately 100 repeats. Table 2 indicates CTG repeat length measurements using PCR for all alleles with <100 repeats (all normal alleles, and expanded alleles in samples NA06075 and NA23265). Repeat lengths for alleles with >100 repeats in Table 2 were calculated using Southern blot analysis. Consensus values were calculated by averaging measurements from each laboratory and calculating the population SD, a measure of how widely values are dispersed from the mean, using Excel software version 14.0.6129.5000 (Microsoft Corp., Redmond, WA). There were no discrepancies among laboratories in the measurements by PCR of the smaller, normal *DMPK* allele in each sample. The DNA size measurements derived from PCR performed by the three laboratories of the mosaic expanded alleles in sample NA06075 were nearly identical. Similarly, laboratories 1 and 3 measured the expanded allele in sample NA23265 using PCR and obtained almost identical repeat lengths (75 and 74, respectively). Laboratory 2 obtained a value of 100 repeats in this sample using Southern blot analysis but did not use PCR because the PCR assay failed to produce a product for this sample.

The three laboratories varied slightly in the measurement of the larger expanded alleles, which were analyzed using Southern blot hybridization, a technique that is inherently less accurate than PCR. The calculated population SD was <10% for all the expanded alleles, except the 2078 repeat alleles in NA03132 (10.4%) and the 377 repeat alleles in NA05164 (14.1%).

Discussion

We identified a set of characterized, cell line–based genomic DNA reference materials for DM1 genetic testing (Table 2). These materials were characterized in three clinical genetic testing laboratories using PCR and Southern blot analysis. The *DMPK* alleles in the samples include four of the five clinical categories: normal (5 to 34 repeats) (10 alleles), mild DM1 (50 to 100 repeats) (three alleles), classical DM1 (101 to 1000 repeats) (five alleles), and congenital DM1 (>1000 repeats) (three alleles).[2] We did not identify any preexisting cell lines in the Coriell collection with repeats in the premutation range (35 to 49 repeats), and neither did we create any lines in this size range from patients in the National Registry. The smallest expanded repeat we identified was 55 repeats (NA06075), which is close to the clinical decision points (35 to 49 CTG repeats). Five new cell lines created from patients with DM1 were expected to have expanded alleles with 90 to 180 CTG repeats; however, each was found to have a much larger expanded allele after cell line creation (Table 1). These patients had reported their CTG repeat size to the Registry based on genetic tests performed 5 to 15 years before the creation of their cell lines for this project, and the CTG repeat size was not measured from the blood samples used to create the cell lines. Previously used methods may have been less accurate, or data transcription errors may have

Table 2 CTG Repeat Length in DNA from 10 DM1 Cell Lines

	Allele 1, 2			
Coriell no.	Laboratory 1	Laboratory 2	Laboratory 3	Consensus size
NA06075*	12, 55, 69 (mosaic)	12, 56, 71 (mosaic)	12, 55, 69 (mosaic)	12, 56, 70 (± 0.9)
NA23265†	12, 75	12, ND‡	12, 74	12, 75
NA23378§	22, 145	22, 135	22, 135	22, 138 (± 5)
NA05164	21, 325	21, 450	21, 357	21, 377 (± 53)
NA03697¶	12, 370	12, 450	12, 417	12, 412 (± 33)
NA04567	21, 630	21, 600	21, 680	21, 637 (± 33)
NA03696	12, 700	12, 680	12, 710	12, 697 (± 13)
NA04648	5, 950	5, 1070	5, 1005	5, 1008 (± 49)
NA05152	5, 1600	5, 1600	5, 1664	5, 1621 (± 30)
NA03132	5, 1950	5, 1900	5, 2384	5, 2078 (± 217)

*Laboratory 3 measured an expanded allele in this sample with 88 CTG repeats using Southern blot analysis.

†Laboratory 2 detected an expanded allele with 100 repeats using Southern blot analysis in this sample. Laboratory 3 measured an expanded allele with 72 repeats using Southern blot hybridization.

‡ND, not determined. Laboratory 2 did not measure the expanded allele in this sample using PCR.

§Laboratory 3 detected two expanded alleles (74 and 125 repeats) in this sample using PCR.

¶Laboratory 2 detected a minor band measuring 530 repeats using Southern blot analysis in this sample.

occurred. CTG repeats in patients with DM1 are known to be unstable, and repeat size has been shown to increase over time.[28] It is possible that the *DMPK* alleles in these patients' peripheral blood cells have expanded significantly since the patients were initially tested or that the samples themselves are unstable as a result of Epstein-Barr virus transformation or clonal expansion during cell culture.

The potential instability of the CTG expansion in these cell lines should not affect the usefulness of these samples as reference materials when isolated genomic DNA is used for testing because several milligrams of genomic DNA were isolated from the cell line. The repeat length in the isolated DNA sample should remain stable over time. In addition, the procedures used by the Coriell Cell Repositories to propagate cell lines are designed to limit the number of cell divisions after establishment of the line, which reduces the possibility of accumulating additional mutations. The standard protocol for banking cell cultures in the Coriell Cell Repositories is to create an initial frozen cell culture stock and to reserve a portion of it for replenishment of frozen cell culture stock and expansion for DNA isolation for distribution. This procedure ensures that distribution stocks are only six or seven population doublings beyond the earliest available stocks and minimizes the opportunity for accumulation of mutations. It is recommended, however, that all users of any reference material should confirm the expected genotype in their own laboratory.[20] We also recommend that owing to the potential instability of the CTG expansion, clinical laboratories use the DNA characterized during this study as reference materials rather than the associated cell lines because the latter have not been evaluated for stability or suitability as reference materials.

The College of American Pathologists proficiency testing survey for DM1 evaluates participants by their ability to correctly interpret their results (consistent or not consistent with DM1) but does not currently grade the reported repeat length measurements [*http://www.cap.org/apps/cap.portal?_nfpb=true&_pageLabel=accreditation* (paid subscription required), last accessed January 11, 2013]. The participants in this program can almost always measure the size of the smaller, normal repeat-length alleles correctly, but there is wide variation in the reported size of the larger, expanded alleles.[29] This variation typically does not affect the participants' ability to identify samples with expanded alleles or to correctly interpret the clinical diagnostic results because the repeat lengths of the diagnostic decision points (35 to 49 repeats) are accurately assigned by PCR findings. Similarly, in this study, the three laboratories were able to consistently measure the smaller alleles; however, they initially reported widely discrepant results for the expanded alleles (data not shown). On reanalysis of the Southern blot data using a common standard of measurement (migration to the midpoint of each band or smear), the repeat size of the expanded alleles reported by each laboratory achieved more concordance. This observation suggests that adoption of a common method for measurement and calculation of expanded allele length will help standardize reporting of the CTG repeat sizes in blood samples of patients with DM1 and may allow proficiency testing programs to evaluate participants based on their reported expanded allele repeat lengths. Use of these characterized genomic DNA samples during assay development and validation can also help laboratories evaluate the capacity of their *DMPK* assays to obtain accurate results.

This study highlights the important role that patient advocacy groups and cell registries, such as the NIH-funded National Registry at the University of Rochester, can have in the improvement of laboratory services and for basic scientific research. Resources, such as publicly available DNA, cell lines, and tissue samples, for rare disorders are limited and may be difficult to obtain. Patient groups are becoming aware of their role in the development of new tests and treatments for their rare disorders and are often active partners with the research and clinical communities. The willingness of patients with DM1 and their families to share their resources with the genetics community through the Coriell Cell Repositories will benefit not only the quality but also the availability of genetic testing for DM1.

The genomic DNA reference materials characterized in this study will be useful for quality control, proficiency testing, test development and validation, and research. DNA samples purified from these cell lines, and other DNA samples characterized by the GeT-RM, are publicly available from the NIGMS repository at the Coriell Cell Repositories. More information about the GeT-RM program and available reference materials are available from the GeT-RM Coordination Program (*http://wwwn.cdc.gov/dls/genetics/RMMaterials*, last accessed January 11, 2013).

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