Journal of Molecular Diagnostics, Vol. 11, No. 6, November 2009
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and the Association for Molecular Pathology
DOI: 10.2353/jmoldx.2009.090078

Development and Characterization of Reference Materials for *MTHFR*, *SERPINA1*, *RET*, *BRCA1*, and *BRCA2* Genetic Testing

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Well-characterized reference materials (RMs) are integral in maintaining clinical laboratory quality assurance for genetic testing. These RMs can be used for quality control, monitoring of test performance, test validation, and proficiency testing of DNA-based genetic tests. To address the need for such materials, the Centers for Disease Control and Prevention established the Genetic Testing Reference Material Coordination Program (GeT-RM), which works with the genetics community to improve public availability of characterized RMs for genetic testing. To date, the GeT-RM program has coordinated the characterization of publicly available genomic DNA RMs for a number of disorders, including cystic fibrosis, Huntington disease, fragile X, and several genetic conditions with relatively high prevalence in the Ashkenazi Jewish population. Genotypic information about a number of other cell lines has been collected and is also available. The present study includes the development and commutability/genotype characterization of 10 DNA samples for clinically relevant mutations or sequence variants in the following genes: *MTHFR*; *SERPINA1*; *RET*; *BRCA1*; and *BRCA2*. DNA samples were analyzed by 19 clinical genetic laboratories using a variety of assays and technology platforms. Concordance was 100% for all samples, with no differences observed between laboratories using different methods. All DNA samples are available from Coriell Cell Repositories and characterization information can be found on the GeT-RM website. *(J Mol Diagn 2009, 11:553–561; DOI: 10.2353/jmoldx.2009.090078)*

The use of genetic tests in medical practice has increased rapidly over the past few years. There are currently over 1400 genetic tests offered in clinical

Supported by a cooperative agreement between the Association for Prevention Teaching and Research and the Centers for Disease Control and Prevention (award number U50/CCU300860); and the Ovarian Cancer SPORE at Fox Chase Cancer Center (P50 CA083638).

Accepted for publication June 10, 2009.

This work was performed at the Centers for Disease Control and Prevention. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Mention of the name of any company or product does not constitute an endorsement by the Centers for Disease Control and Prevention. The Centers for Disease Control and Prevention is not responsible for the validity of commercially or publically available products mentioned in this publication.

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Table 1. Publicly Available Higher Order RMs

Organization	CRM/SRM	FDA-cleared*	CE marked*†
NIBSC‡	World Health Organization Reference Reagent Factor V Leiden, Human gDNA World Health Organization Reference Reagent Prothrombin Mutation G20210A, Human gDNA		
IRMM§	Plasmid DNA carrying human prothrombin gene (G2021A mutation) CRM Plasmid DNA carrying human prothrombin gene (Wildtype) CRM Plasmid DNA carrying human prothrombin gene (heterozygous for G2021A mutation)		
NIST¶	SRM Mitochondrial DNA Sequencing (Human HL-60 DNA) SRM Mitochondrial DNA Sequencing (Human DNA) SRM Fragile X Human DNA Triplet Repeat Standard SRM Heteroplasmic Mitochondrial DNA Mutation Detection Standard		
ParagonDx‖		CYP2D6 *4a/*2AXN CYP2D6 *2 mol/L/*17 CYP2D6 *29/*2AXN CYP2D6 *6B/*41 CYP2D6 *1/*5 CYP2D6 *3A/*4A	
Maine Molecular Quality Controls**		INTROL Cystic Fibrosis Panel I	

*Information provided by manufacturer.
†It is important to note that while higher order RMs are available for a small number of diseases, CE-marked RMs are currently unavailable.
‡For more information, please refer to the National Institute for Biological Standards and Controls (NIBSC) website (*http://www.nibsc.ac.uk*, last accessed December 19, 2008).
§For more information, please refer to the Institute for Reference Materials and Measurements (IRMM) website (*http://irmm.jrc.ec.europa.eu/html/homepage.htm*, last accessed December 19, 2008).
¶For more information, please refer to the NIST website (*http://ts.nist.gov/measurementservices/referencematerials/index.cfm*, last accessed December 19, 2008).
‖ParagonDx (Morrisville, NC).
**Maine Molecular Quality Controls (Scarborough, ME).

laboratories and hundreds of additional tests are available in research settings (National Institutes of Health, *http://www.genetests.com*, last accessed March 2, 2009). As for all medical testing, laboratories performing genetic tests must adhere to established quality assurance practices to ensure confidence in test integrity and accuracy.[1] An integral part of quality assurance is the use of characterized and readily available reference materials (RMs). RMs, such as characterized DNA or cell lines that have a defined property, such as commutability (the equivalency of results between different measurement procedures) or genotype, can be used for quality control, monitoring of test performance, detection of errors, and proficiency testing of DNA-based genetic tests.[1–3]

A hierarchy of RMs has previously been described based on the degree of characterization of each material.[3–6] The top hierarchy includes Standard Reference Materials (SRMs), which are produced by the National Institute of Standards and Technology (NIST); Certified Reference Materials (CRMs), produced by several organizations including the Institute of Reference Materials and Measurements; and other materials available from independent organizations, such as the World Health Organization. These RMs possess properties that are certified by a procedure that establishes metrological traceability and degree of uncertainty.[3–6] These materials have also been extensively characterized to ensure their homogeneity and stability. On the next level of the hierarchy are those RMs whose properties are sufficiently homogeneous and established for use in quality control applications.[4] These materials include some commercially available RMs, which have either been cleared by the US Food and Drug Administration (FDA) or CE-marked (a mandatory conformity mark for products placed within the European Union market), and are therefore cleared for *in vitro* diagnostic use. For the purposes of this article, we will call SRMs, CRMs, FDA-approved, and CE-marked RMs "higher order" RMs. The use of "higher order" in this article does not represent a technical definition but only a categorization. Some manufacturers and governmental organizations have developed higher order RMs for a small number of diseases (Table 1), but many more are needed. In the absence of widely available higher order RMs, laboratories must use other types of materials as controls such as residual patient specimens, which are not reliably available or renewable. Other materials exist such as genomic DNA or cell lines that are available from sources such as repositories. Ideally these renewable materials are characterized by DNA sequence analysis or evaluated in multiple independent laboratories.[5] Though not higher order RMs, these genomic DNA or cell line materials play a vital role in quality control by providing a publicly available, renewable, and inexpensive source of RMs for various laboratory quality assurance needs.

To address the need for improved publicly available characterized RMs for genetic testing (on all levels of the RM hierarchy), the Centers for Disease Control and Prevention (CDC), the National Institutes of Health, and NIST held a series of meetings to discuss possible solutions.[6] Based on resulting recommendations, the CDC established the Genetic Testing Reference Material (GeT-RM) Coordination Program, which coordinates a process to improve the availability of appropriate RMs for the genetic testing community (GeT-RM Program, *http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx*, last accessed March 2, 2009). Though sponsored by the CDC, much of the work performed by the GeT-RM, including RM priority decisions, specimen collection, material development, and molecular genetic characterization, occurs through voluntary collaborations with various clinical genetic laboratories. To date, the GeT-RM program has coordinated the development and/or commutability/genotype characterization of RMs for cystic fibrosis,[7] Huntington disease,[8] fragile X syndrome,[9] and several genetic conditions with relatively high prevalence in the Ashkenazi Jewish population[10]; and has also provided information for several pharmacogenetic markers, including members of the *CYP450* gene family, *VKORC1*, and *UGT1A1.*

In this study, the GeT-RM coordinated RM characterization studies of 10 genomic DNA samples containing sequence changes associated with hyperhomocysteinemia (*MTHFR*), and clinically relevant gene mutations in α-1 antitrypsin deficiency (*SERPINA1*), multiple endocrine neoplasia type 2A (*RET*), and hereditary breast and ovarian cancer (*BRCA1* and *BRCA2*). The genomic DNA samples used in this study were characterized by using a variety of assays in a total of 18 College of American Pathologists-accredited and Clinical Laboratory Improvement Amendment-certified laboratories performing clinical genetic testing. These samples are publicly available from the Coriell Cell Repositories (Camden, NJ) and can be used for various quality assurance purposes and for research.

Materials and Methods

Mutation Selection

These specific loci were chosen for this study because cell lines containing these clinically relevant mutations were recently made available at the Coriell Cell Repositories. Because of their clinical relevance, the GeT-RM aimed to conduct characterization studies on them and make these data publicly available in a timely fashion, which is why they are grouped together in a single study.

The *MTHFR* gene encodes a key enzyme in homocysteine metabolism. Certain sequence variants in this gene (677C>T and 1298A>C) have been associated with hyperhomocysteinemia, which is characterized by abnormally high levels of homocysteine in the blood.[11–15] For this study, one DNA sample containing a heterozygous 677C>T change and a homozygous 1298A>C variant was characterized.

Specific mutations in *SERPINA1* lead to various levels of α-1 antitrypsin deficiency (AATD), which is characterized by pulmonary disease in adults and liver disease in children and adults (National Institutes of Health, *http://www.genetests.com*, last accessed March 2, 2009).[16] The normal allele, M, produces normal levels of the AAT protein. Two mutations, S and Z (representing the Glu264Val and Glu342Lys mutations, respectively), produce low or very low levels of circulating AAT protein, respectively, and patients carrying two of these alleles (ZZ or SZ) are at risk for developing disease related to AATD. Individuals who are MZ, MS, or SS produce enough AAT to function normally.[16–19] Four genomic DNA samples with the MS, MZ, SZ, and ZZ genotypes were included in this study, covering both at-risk and nonrisk testing.

Mutations in *RET* can lead to multiple endocrine neoplasia type 2A (MEN2A), MEN 2B and familial medullary thyroid carcinoma (National Institutes of Health, *http://www.genetests.com*, last accessed March 2, 2009).[20–22] Mutations in cysteine residues at codons 609, 611, 618, 620, and 634 account for 95% of families with MEN2A and 85% of families with familial medullary thyroid carcinoma.[23–25] Two genomic DNA samples were included in this study, one heterozygous for the Cys620Phe mutation and one heterozygous for the Cys618Ser mutation.

Women who inherit certain *BRCA1* or *BRCA2* mutations have an increased risk of developing breast and ovarian cancer compared with the general population.[26] Mutations in these two genes are also associated with an increased risk for prostate, pancreatic, and colon cancer (National Institutes of Health, *http://www.genetests.com*, last accessed March 2, 2009).[27–29] Three Ashkenazi Jewish founder *BRCA* mutations, 185delAG and 5382insC (in *BRCA1*), and 6174delT (in *BRCA2*), which account for 90% of breast and ovarian cancer in Ashkenazi Jewish patients, were included in this study.[30–32]

Cell Line Creation and DNA Preparation

Seven cell lines used in this study were previously available from the Coriell Cell Repositories and were selected based on the description of genotype in the catalogue. Two of these lines were reported to contain mutations in *SERPINA1* (GM03578 and GM03579), two contained mutations in *RET* (GM16658 and GM16660), two contained mutations in *BRCA1* (GM13715 and GM14090), and one contained a *BRCA2* mutation (GM14170). The only genotype information available for these cell lines was information obtained from the original submitter of the materials to the repository. The cell lines were selected for study based on this reported genotype. The reported genotype of each line was confirmed in each case. Three additional cell lines, one carrying a sequence change in *MTHFR* (GM20730) and two carrying *SERPINA1* mutations (GM20835 and GM20918), were created specifically for this project from residual patient specimens tested in a clinical laboratory as follows: after mutation identification, residual whole blood was sent to the Coriell Institute, under an existing institutional review board protocol, for B-lymphocyte transformation by Epstein-Barr virus as previously described.[33,34] All 10 cell lines were cultured and expanded in antibiotic-free media. Cell suspensions were aliquoted in 1-ml vials containing approx-

Table 2. Investigators and Assays Used

Laboratory	*MTHFR*	*SERPINA1* (AATD)	*RET* (MEN2)	*BRCA1/BRCA2*
1	PCR/Luminex	PCR/Luminex		
2		PCR with hybridization probes and melting curve analysis	DNA sequence analysis	
3			DNA sequence analysis	
4	PCR with hybridization probes and melting curve analysis			
5			DNA sequence analysis	
6			DNA sequence analysis	
7		PCR/RE/electrophoresis		DNA sequence analysis
8				DNA sequence analysis
9			DNA sequence analysis	Allele-specific oligonucleotide hybridization
10	Third Wave Technologies Invader ASR			
11	Autogenomics INFINITI Assay			
12		PCR/RE/ABI3100		
13	Third Wave Technologies Invader ASR			
14				Heteroduplex mobility assay
15	PCR with hybridization probes and melting curve analysis DNA sequence analysis	PCR with hybridization probes and melting curve analysis		
16		PCR/RE/electrophoresis DNA sequence analysis		
17		Mutliplex allele-specific PCR/ electrophoresis		
18	PCR/RE/electrophoresis			DNA sequence analysis

RE, restriction enzyme analysis; ASR, analyte specific reagent; ABI3100, Genetic Analyzer 3100 (Applied Biosystems, Foster City, CA).

imately 5×10^6 viable cells per vial. Cultures were cryopreserved in heat-sealed borosilicate glass ampoules and stored in liquid nitrogen. Successful cultures were bacterial, fungal, and mycoplasma-free and capable of doubling in cell number within 4 days of recovery. Coriell prepared approximately 2 mg of DNA from each cell line in this study by using Gentra/Qiagen Autopure (Valencia, CA) per manufacturer's instructions or as previously described.[35]

Laboratory Participation

Clinical laboratories were solicited for participation based on their capability to detect the mutations included in this study and on their current assay methods. This ensured that each of the DNA samples was tested in multiple laboratories using a variety of the commonly used assays and technology platforms. A total of 18 clinical genetic laboratories agreed to participate in this study. Table 2 shows the gene(s) for which individual laboratories performed testing and the assay(s) or platform(s) they used. All laboratories are located within the United States and are accredited by the College of American Pathologists and Clinical Laboratory Improvement Amendment certified.

Protocol

Each of the 18 laboratories received a 50-μg aliquot of extracted DNA from each cell line they were asked to test. With the exception of those laboratories performing DNA sequence analysis, the expected mutation(s) for each DNA sample were not disclosed. Laboratories assayed each DNA sample by using their routine clinical assays. Results were sent directly to study coordinators (L.V.K. and S.D.B.), who reviewed the data for quality and discrepancies.

Assays/Platforms Used

MTHFR

A total of eight laboratories tested for nucleotide sequence variants in *MTHFR*. One laboratory used a commercial kit (INFINITI Assay, Autogenomics, Carlsbad, CA) and followed manufacturer's instructions. Two laboratories incorporated a commercial analyte-specific reagent (*MTHFR* 677 and *MTHFR* 1298, Third Wave Technologies, Madison, WI) into their laboratory developed test (LDT).[36] Three additional laboratories also used a LDT. One laboratory used PCR and target-specific extension with the Luminex liquid chip decoding system (Luminex Corporation, Austin, TX), one laboratory used real-time PCR with fluorescent hybridization probes followed by melting curve analysis, and one laboratory used PCR followed by restriction enzyme analysis and agarose gel electrophoresis (restriction fragment length polymorphism). An additional laboratory used two methods for detection, PCR with hybridization probes followed by melting curve analysis and bi-directional sequencing with Big Dye Terminator chemistry and the ABI3100

Genetic Analyzer (Applied Biosystems, Foster City, CA) for confirmation.

SERPINA1

Eight laboratories tested for the *SERPINA1* mutations, with seven performing their own LDT and one performing partial sequencing of exons 3 and 5 on an ABI 3100 Genetic Analyzer using Big Dye Terminator Cycle Sequencing kits (Applied Biosystems). One LDT used PCR and target-specific extension with the Luminex liquid chip decoding system (Luminex Corporation). Two laboratories used PCR with hybridization probes followed by melting curve analysis. Three laboratories used PCR followed by restriction enzyme analysis, two of which used agarose gel electrophoresis to analyze the results[37–40] and the other (whose PCR primers were labeled with 6-FAM) used the ABI 3100 Genetic Analyzer (Applied Biosystems) for capillary electrophoresis. The last LDT used multiplex allele-specific PCR for M, S, or Z mutations followed by agarose gel electrophoresis.[37,41,42]

RET

Five laboratories tested for *RET* proto-oncogene mutations. Three laboratories sequenced exons 10, 11, 13, 14, 15, and 16 with bi-directional Sanger sequencing using Big Dye Terminator chemistry on the ABI 3730 and ABI 3130xl analyzers (both from Applied Biosystems).[43,44] A fourth laboratory sequenced exons 10, 11, 13, and 14 using Big Dye Terminator chemistry and the ABI3130xl Genetic Analyzer. This same laboratory used PCR and restriction fragment length polymorphism for detection of the *MEN2B* mutation in exon 16. The fifth laboratory used proprietary sequencing methods to sequence exons 10, 11, 13, and 14.

BRCA1/BRCA2

A total of five laboratories tested DNA samples for mutations in *BRCA1* and *BRCA2*. Three laboratories performed DNA sequence analysis for the relevant regions of *BRCA1* and *BRCA2*, by using Big Dye Terminator chemistry and capillary electrophoresis, with two laboratories using the ABI3130 Genetic Analyzer and one using the ABI3130xl Genetic Analyzer (both from Applied Biosystems). The remaining two laboratories used a LDT for *BRCA1* and *BRCA2* mutation identification. In one LDT, allele-specific oligonucleotides were hybridized with multiplex amplified product containing *BRCA1* exons 2 and 20 along with the relevant portion of *BRCA2* exon 11. Mutations were detected via chemiluminescent-labeled probes for normal and mutant sequences by using allele-specific oligonucleotide analysis. The other LDT used a heteroduplex mobility assay in which PCR products for the individual exons were separated by using pre-cast gels (Invitrogen, Carlsbad, CA) for exon 2 (173 bp), exon 11 (98 bp), or exon 20 (275 bp). Gels were stained with ethidium bromide and any variant detected was confirmed by direct sequencing.[45–47]

Results

A panel of genomic DNA reference materials containing sequence variants deemed clinically relevant and/or frequently ordered by clinicians was produced based on discussions with clinical laboratory geneticists. These include sequence variants in *MTHFR* and mutations in *SERPINA1*, *RET*, *BRCA1*, and *BRCA2*. Seven cell lines containing the appropriate mutations were previously available at Coriell Cell Repositories and three additional cell lines, two carrying mutations in *SERPINA1* (GM20835 and GM20918) and one carrying *MTHFR* variants (GM20730), were created specifically for this study.

The methods used in this project were representative of the most commonly used assay technologies and instrument platforms in clinical laboratories. With the voluntary participation of 18 laboratories, we were able to include a wide variety of assay methods, both commercially available and laboratory-developed.

The genotype of each DNA sample, the different testing assays or platforms used, and the number of laboratories using each platform are summarized in Table 3. Concordance was 100% for all samples included in this project. No differences were observed between laboratories using different assay methods or platforms. In addition, no false-positive or other discordant results were reported among the laboratories. All cell lines and DNA products characterized in this study are publicly available from the Coriell Cell Repositories. The colloquial names and corresponding Human Genome Variation Society nomenclature of each of the mutations included in this study are listed in Table 4.

Discussion

The GeT-RM program endeavors to improve the availability of characterized RMs for genetic testing. In this study, 10 genomic DNA samples with 10 different clinically important genotypes in five genes (*MTHFR*, *SERPINA1*, *RET*, *BRCA1*, and *BRCA2*) were characterized by 18 separate clinical genetic laboratories using a variety of assays and technology platforms. No discordant results were reported and there were thus no differences between laboratories using different assay platforms.

In this study, we were able to test DNA from a cell line carrying both a homozygous 1298A>C mutation and a heterozygous 677C>T mutation in the *MTHFR* gene. This genotype has been previously reported by Brown et al.[15] Since this DNA represented one of the possible genotypes that could be tested in a clinical laboratory, it was included in the study. For α-1-antitrypsin deficiency, a total of four different genotypes were included in this study, MZ, ZZ, MS, and SZ, covering both at-risk and nonrisk genetic testing.[16–19] Through this study, we were also able to develop and add the SZ and MS *SERPINA1* genotypes to the Coriell collection (which were previously missing). For multiple endocrine neoplasia type 2A, we were able to test two *RET* genotypes, C618S and C620F. These mutations, along with mutations in codons 609 and 611 make up 15% of MEN2A mutations. Mutations in

Table 3. Results of Multi-Laboratory Characterization Studies

		Confirmed genotype		Coriell catalog info.		Characterization methods	
Disorder	Gene	Allele 1*	Allele 2*	Cell line number	DNA number	Sequencing, No. of labs	Other methods† (No. of labs)
Hyperhomocysteinemia	*MTHFR*	677C>T 1298A>C	1298A>C	GM20730	NA20730	1	A(1), B(2), C(2), D(1), E(1)
AATD	*SERPINA1*	MS‡		GM20835	NA20835	1	A(1), B(2), E(2), F(1), G(1)
		SZ‡		GM20918	NA20918	1	A(1), B(2), E(2), F(1), G(1)
		ZZ‡		GM03578	NA03578	1	A(1), B(2), E(2), F(1), G(1)
		MZ‡		GM03579	NA03579	1	A(1), B(2), E(2), F(1), G(1)
MEN2A	*RET*	C620F		GM16658	NA16658	5	
		C618S		GM16660	NA16660	5	
Various cancers	*BRCA1*	5382insC		GM13715	NA13715	3	H(1), I(1)
		185delAG		GM14090	NA14090	3	H(1), I(1)
Various cancers	*BRCA2*	6174delT		GM14170	NA14170	3	H(1), I(1)

*Allele names given here are colloquial. For proper mutation nomenclature, please refer to Table 4 and the Human Genome Variation Society website (*http://www.hgvs.org/mutnomen*, last accessed December 19, 2008).

†Methods: (A) PCR/Luminex; (B) PCR with hybridization probes and melting curve analysis; (C) Third Wave Technologies Invader Analyte Specific Reagent (ASR); (D) Autogenomics INFINITI assay; (E) PCR/restriction enzyme digest/electrophoresis; (F) PCR/restriction enzyme digest/ABI3100; (G) Multiplex allele-specific PCR/electrophoresis; (H) Allele-specific oligonucleotide hybridization; (I) Heteroduplex mobility assay.

‡ The letters M, S, and Z were originally used to designate the protein, a node to cathode, in isoelectric focusing (see Fagerhol and Braend[53]). M represents a wildtype allele, S represents the mutation NM_00295:c.863A>T (p.Glu288Val), and Z represents the mutation NM_00295:c.1096G>A (p.Glu366Lys).

codon 634 make up 85% and presently RMs for this mutation are not currently available and need to be developed.[23–25] The GeT-RM is presently working on obtaining such RMs as well as those for mutations in codons 609 and 611. Finally, we tested three *BRCA* Ashkenazi Jewish founder mutations, 5382insC, 185delAG, and 6174delT, which account for 90% of breast and ovarian cancer in Ashkenazi Jewish women and are the first line of genetic testing for this population.[30–32]

These characterized genomic DNA RMs, are useful as a component of a quality management plan, including proficiency testing, test development, test validation, quality control, and research. It should be noted that these materials were characterized for genotype and no stability or homogeneity studies were performed. The National Institute of General Medical Sciences Human Genetic Cell Repository in the Coriell Cell Repositories has been ISO (International Organization for Standardization) 9001-certified since 2004 and has been distributing high quality DNA since 1989. The repository has prepared DNA samples for the multiple-laboratory verification of genotype and commutability since 2001. The present study demonstrates how characterization studies for these publicly available materials add even more value for use in laboratory quality assurance. It should also be noted that having different cell lines with the same allelic combinations would be useful for both validation studies and for proficiency testing. While this study focuses primarily on developing and characterizing RMs to fill the need of the genetics community who are lacking RMs for certain clinically relevant mutations, future studies by the GeT-RM and others could focus on character-

Table 4. Nomenclature of Mutations

		Colloquial name		Human Genome Variation Society nomenclature*	
Disease	Gene	Allele 1	Allele 2	Allele 1	Allele 2
Hyperhomocysteimia	*MTHFR*	677C>T	1298A>C	NM_005957.3:c.665C>T	NM_005957.3:c.1286A>C
		1298A>C		NM_005957.3:c.1286A>C	
AATD	*SERPINA1*	MS†		Wildtype	NM_00295:c.863A>T
		SZ†		NM_00295:c.863A>T	NM_00295:c.1096G>A
		ZZ†		NM_00295:c.1096G>A	NM_00295:c.1096G>A
		MZ†		Wildtype	NM_00295:c.1096G>A
MEN2A	*RET*	C620F		NM_020975.4:c.2049G>T	
		C618S		NM_020975.4:c.2043G>C	
Various cancers	*BRCA1*	5382insC		NC_000017.9:g.38529572_38529571delAG	
		185delAG		NC_000017.9:g.38462606dupC	
Various cancers	*BRCA2*	6174delT		NC_000013.9:c.24822delT	

*For more proper mutation nomenclature guidance, please refer to the Human Genome Variation Society website (*http://www.hgvs.org/mutnomen*, last accessed December 19, 2008).

†The letters M, S, and Z were originally used to designate the protein, anode to cathode, in isoelectric focusing (see Fagerhol and Braend[53]). M represents a wildtype allele, S represents the mutation NM_00295:c.863A>T (p.Glu288Val), and Z represents the mutation NM_00295:c.1096G>A (p.Glu366Lys).

izing multiple cell lines for one mutation to further assist in improving genetic testing quality.

These DNA samples, as well as the cell lines from which they were derived, are available at Coriell Cell Repositories. The mutation characterization information accumulated in this study can be found on the GeT-RM website (GeT-RM Program, *http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx*, last accessed March 2, 2009) as well as the Coriell website (Coriell Cell Repositories, *http://ccr.coriell.org/*, last accessed March 12, 2009).

Access to well-characterized RMs is important to ensure the quality of genetic testing. Regulatory requirements, such as the Clinical Laboratory Improvement Amendment, as well as most professional guidelines, including those issued by the College of American Pathologists, the American College of Medical Genetics, the Association for Molecular Pathology, and the European Molecular Genetics Quality Network, recommend the use of RMs when available for quality control, quality assurance, and test development and validation (College of American Pathologists, *http://www.cap.org*, last accessed August 1, 2008; American College of Medical Genetics, *http://www.acmg.net/Pages/ACMG_Activities/stds-2002/g.htm*, last accessed August 1, 2008; European Molecular Genetics Quality Network, *http://www.emqn.org/emqn/BestPractice.html*, last accessed August 1, 2008).[6,48–51]

In an ideal environment, the genetic testing community would have at its disposal higher order RMs such as CRMs, SRMs, and FDA approved/CE-marked products for all genetic tests. However, although these higher order RMs are preferred by laboratorians and test developers, they are currently available for only a few genetic tests, including Factor V Leiden, Prothrombin 20210G>A, Fragile X triplet repeat sizing, *CYP2D6* polymorphism analysis, cystic fibrosis carrier screening, and mitochondrial DNA mutation detection (Table 1). These materials represent only 0.35% of the 1413 genetic tests currently available in clinical practice (GeneTests, *http://www.genetests.org/*, last accessed March 2, 2009). Also, these higher order RMs are typically expensive and only sold in small quantities.

The lack of available higher order and other well characterized RMs presents challenges for many aspects of test development and quality assurance. Laboratories and commercial test developers currently need large numbers of samples to develop and validate new LDTs and potential commercial tests, and for 510(k) submission to the FDA. Also, because higher order RMs are generally available in limited quantities, laboratories also need more widely available materials that can be used on a daily basis for a variety of purposes, including quality control. Characterized genomic DNA samples from repositories, such as those described in this and other GeT-RM studies provide a useful laboratory resource. As with all reagents used in the clinical setting, laboratories must validate the genotype of these materials before use (College of American Pathologists, *http://www.cap.org*, last accessed August 1, 2008).[48,50]

The genetics community has identified the lack of available quality control and RMs for genetic testing as a critical need for laboratory test development and quality assurance.[52] To address this need, the GeT-RM Program, together with the genetics community, is working to create and characterize publicly available, renewable sources of genomic DNA useful as RMs.

The GeT-RM has developed and/or characterized (for genotype) over 85 genomic DNA samples derived from cell lines. These include materials for various inherited genetic disorders, such as cystic fibrosis,[7] Huntington disease,[8] Fragile X,[9] and genetic conditions with relatively high prevalence in the Ashkenazi Jewish population[10] (for example, Bloom syndrome, Canavan disease, Fanconi anemia, Tay Sachs disease, familial dysautonomia, Gaucher disease, glycogen storage disease type 1a, Mucolipidosis IV, and Niemann-Pick disease). The program is currently developing new cell lines and conducting RM (on the lower level of the hierarchy) commutability and genotype characterization studies for Duchenne muscular dystrophy, pharmacogenetic loci and disorders included in the American College of Medical Genetics newborn screening panel. In addition to coordinating characterization studies, the GeT-RM has also collected genetic data from other sources on over 250 additional materials, including a number of cell lines with clinically important polymorphisms in the pharmacogenomic loci *CYP450* (2D6, 2C9, and 2C19), *VKORC1*, and *UGT1A1*. Data on all of these materials are available on the GeT-RM website (GeT-RM Program, *http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx*, last accessed March 2, 2009).

The GeT-RM process is not meant to circumvent the development of higher order RMs. Indeed, the GeT-RM actively encourages the use of these materials by testing laboratories when available and promotes the development of higher order RMs by commercial manufacturers and other RM producers.

Although residual patient samples, excess proficiency testing materials, or material available from various repositories, including the characterized genomic DNA samples described in this study, are not optimal RMs for quality assurance purposes, they can be used in a responsible and appropriate manner by laboratories and assay developers. These RMs can also be used to supplement available higher order RMs for daily use. The GeT-RM is working to bridge the gap by providing readily available well-characterized genomic RMs to fill an urgent unmet need while simultaneously advocating for the development and use of higher order RMs for genetic testing. More information about the GeT-RM and its work can be found on their website (GeT-RM Program, *http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx*, last accessed March 2, 2009).

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