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Characterization of 107 Genomic DNA Reference Materials for *CYP2D6*, *CYP2C19*, *CYP2C9*, *VKORC1*, and *UGT1A1*

A GeT-RM and Association for Molecular Pathology Collaborative Project

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Pharmacogenetic testing is becoming more common; however, very few quality control and other reference materials that cover alleles commonly included in such assays are currently available. To address these needs, the Centers for Disease Control and Prevention's Genetic Testing Reference Material Coordination Program, in collaboration with members of the pharmacogenetic testing community and the Coriell Cell Repositories, have characterized a panel of 107 genomic DNA reference materials for five loci (*CYP2D6*, *CYP2C19*, *CYP2C9*, *VKORC1*, and *UGT1A1*) that are commonly included in pharmacogenetic testing panels and proficiency testing surveys. Genomic DNA from publicly available cell lines was sent to volunteer laboratories for genotyping. Each sample was tested in three to six laboratories using a variety of commercially available or laboratory-developed platforms. The results were consistent among laboratories, with differences in allele assignments largely related to the manufacturer's assay design and variable nomenclature, especially for *CYP2D6*. The alleles included in the assay platforms varied, but most were identified in the set of 107 DNA samples. Nine additional pharmacogenetic loci (*CYP4F2*, *EPHX1*, *ABCB1*, *HLAB*, *KIF6*, *CYP3A4*, *CYP3A5*, *TPMT*, and *DPD*) were also tested. These samples are publicly available from Coriell and will be useful for quality assurance, proficiency testing, test development, and research. ***(J Mol Diagn 2010, 12:835–846; DOI: 10.2353/jmoldx.2010.100090)***

Many laboratories are testing for pharmacogenetic (PGx) markers, common genetic variants that are usually considered only when a patient is likely to be exposed to a

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Standard of practice is not being defined by this article, and there may be alternatives.

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particular pharmaceutical. There are, however, very few publicly available characterized quality control and other reference materials (RMs) for PGx markers to assure the quality of testing. These materials are necessary for test development, test validation, quality control, and proficiency testing. Moreover, the available materials do not include many of the alleles tested in laboratory PGx assays. Publicly available cell lines may be used as RMs, but few have been characterized for multiple PGx loci and multiple alleles.

The clinical laboratory testing community has expressed a desire for RMs that are characterized for multiple PGx loci. To address these needs, the Centers for Disease Control and Prevention (CDC)-based Genetic Testing Reference Material Coordination Program (GeT-RM, *http://wwwn.cdc.gov/dls/genetics/rmmaterials/default.aspx*, last accessed on June 16, 2010) in collaboration with the Association for Molecular Pathology, members of the pharmacogenetic testing community and the Coriell Cell Repositories (Camden, NJ), have characterized a panel of 107 genomic DNA RMs for five loci (*CYP2D6*, *CYP2C19*, *CYP2C9*, *VKORC1*, and *UGT1A1*) commonly included in PGx testing panels and proficiency testing surveys. 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.

Pharmacogenetic testing encompasses molecular testing for common genetic variants associated with human variability in drug response. There are a considerable number of polymorphisms in many of the human genes associated with pharmacokinetics or pharmacodynamics of exogenous drugs. Pharmacogenetic sequence variants are designated by a nomenclature of *(star) alleles, where *1 is designated as normal (commonly referred to as wild-type or fully functional) and subsequent variant alleles are numbered in the order that they are identified and characterized. Within each '*' allele designation, additional variations that are linked in *cis* with the defining single nucleotide polymorphism (SNP) create subfamilies that are designated alphabetically in the order that they are described (eg, *2A, *2B, *2C). Unlike many heritable disease mutations, each PGx allele may include several SNPs, rather than a single site mutation. The PGx nomenclature adds to the analytic and reporting complexity, especially when trying to compare various assay platforms. PGx gene variants typically characterize drug metabolizing enzymes, drug transporters, drug receptors, or targets of drug action.

The human liver is the primary site responsible for processing and transforming a variety of toxins and other compounds, including pharmaceuticals, into active or inactive metabolites. This activity is mediated by dozens of metabolic enzymes with a wide range of biochemical activities. Cytochrome P450 (CYP450) is a superfamily of liver enzymes that catalyzes the conversion of substances that are excreted from the body. The CYP450s metabolize substrates through a variety of reactions (including epoxification, N-dealkylation, O-dealkylation, S-oxidation and hydroxylation). The substrates include not only drugs and hormones but also food and dietary components, occupational pollutants, and industrial chemicals. More than half of all drugs are primarily metabolized by the CYP450 enzymes.[1] Fifty-seven cytochrome P450 enzymes have been identified in humans, 15 of which are involved in metabolism of xenobiotic compounds, including pharmaceuticals.[1] The activity of each enzyme encoded by the combination of cytochrome P450 *alleles is categorized as one of four possible phenotypes: extensive (normal) metabolizer, intermediate metabolizer, poor metabolizer, and ultra-rapid metabolizer. There is some variability in the phenotypes conferred by each genetic variant that depend on the target substrate.

CYP2D6 is one enzyme in the CYP450 superfamily estimated to metabolize approximately 25% of all clinically used drugs.[2] Some pharmaceuticals metabolized by CYP2D6 include the following: cancer drugs such as tamoxifen, antidepressants such as fluoxetine and amitriptyline, and narcotic analgesics such as codeine. The *CYP2D6* gene, located on chromosome 22, is highly polymorphic. Currently there are more than 75 alleles described for *CYP2D6* (Human Cytochrome P450 [CYP] Allele Nomenclature Committee *http://www.cypalleles.ki.se/cyp2d6.htm*, last accessed on February 11, 2010). Each allele has varying frequencies based on the ethnic background of the patient population.[3,4]

CYP2C19, another member of the cytochrome P450 family, is important for the metabolism of a number of hormones and prescription drugs including omeprazole, clomipramine, and clopidogrel. A variety of alleles, including the common nonfunctional (poor metabolizing) *2 and *3 variants, as well as an ultra-rapid metabolizing *17 variant, exist for this enzyme (Human Cytochrome P450 [CYP] Allele Nomenclature Committee *http://www.cypalleles.ki.se/cyp2c19.htm*, last accessed on December 15, 2009). The frequency of these alleles varies among different ethnic populations.[3] The FDA changed the boxed warning label on Plavix (clopidogrel), to recommend *CYP2C19* testing to identify poor metabolizers (*http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm203888.htm*, last accessed on June 15, 2010).

Inconsistency in response to the anticoagulant warfarin is affected by genetic variability in the enzymes CYP2C9, which metabolizes warfarin and Vitamin K epoxide reductase complex subunit 1 (VKORC1), the target of warfarin action. Warfarin has a narrow therapeutic index, variable dosing requirements, and necessitates frequent monitoring of prothrombin time to achieve safe and effective reduction of thrombotic risk. Testing for genetic differences that contribute to variable warfarin response can aid in the clinical management of patients receiving warfarin therapy, although this has been controversial.[5] *CYP2C9* and *VKORC1* genotyping can be used to guide dosing and may help minimize adverse drug reactions such as bleeding from excess administration of anticoagulants and thrombosis caused by underdosing.[6] Commonly tested alleles include *CYP2C9* *2 and *3 (slow metabolizing alleles) (Human Cytochrome P450 [CYP] Allele Nomenclature Committee *http://www.cypalleles.ki.se/cyp2c9.htm*, last accessed on February 11, 2010), and *VKORC1* c.1639G>A, all of which are associated with increased sensitivity to warfarin.[7]

Table 1. Loci and Alleles Detected by Each Assay[†]

CYP2D6	*CYP2C19*	*CYP2C9*	*VKORC1*	*UGT1A1*
Roche Amplichip (FDA-cleared) *2–*11, *15, *17, *19, *20, *29, *35, *36, *40, *41, *1XN, *2XN, *4XN, *10XN, *17XN, *35XN, *41XN (sets 1, 2, 3)[‡]	Roche Amplichip (FDA-cleared) *2–*3 (sets 1, 2, 3)	AutoGenomics INFINITI (RUO) *2–*6, *11 (sets 1, 2, 3)	AutoGenomics INFINITI (RUO) 3673, 5808, 6009, 6484, 6853, 7566, 8773, 9041 (sets 1, 2, 3)	AutoGenomics INFINITI (RUO) *28, *36, *37 (sets 1, 2, 3)
AutoGenomics INFINITI (RUO) *2–*10, 12, *14, *17, *29, *41, *XN (sets 1, 2, 3)	AutoGenomics INFINITI (RUO) *2–*10, *17 (sets 1, 2, 3)	Idaho Tech (LDT with ASRs) *2, *3 (sets 1, 2, 3)	Idaho Tech (LDT with ASR) 3673 (sets 1, 2, 3)	PCR/CE (LDT) ***28, *36, *37 (set 3)**
Luminex (IUO) *2–*12, *14, *15, *17, 41, *1XN, *2XN, *4XN, *10XN, *17XN, *41XN (sets 1, 2, 3)	RFLP (LDT) *2, *3 (set 1)	TaqMan (LDT) *2, *3, *5, *6, *8, *9, *11, *12 (set 1)	Luminex (LDT) 3673 (set 1, 2)	PCR/CE (LDT) *28,*36, *37 (set 2)
ParagonDx (RUO) *2–*7, *10, *17, *29, *35, *41, *1XN, *2XN, *4XN (set 1)	TaqMan (LDT) *2–*9, *12 (set 1)	Luminex (LDT) *2, *3 (sets 1, 2)	SNaPShot (LDT) 3673 (sets 1, 2, 3)	PCR/CE (LDT) *28, *36, *37 (set 1)
LDT SNaPShot (LDT) *2-*8, *10, *17, *2XN (set 1)	Luminex (IUO) *2–*8 (sets 1, 2, 3)	SNaPShot (LDT) *2, *3, *5, *6 (sets 1, 2, 3)	Osmetech eSensor (FDA-cleared) 3673 (sets 1, 2, 3)	Hologic, Inc. Invader (FDA-cleared) *28, *36, *37 (sets 1, 2, 3)
		Osmetech eSensor (IUO) *2, *3, *5, *6, *11, *14–16 (sets 1, 2, 3)		

[†] All assays assume *1 if no mutations are detected.
[‡] Indicates sample set(s) tested.
LDT, laboratory-developed test; RUO, research use only; IUO, investigational use only; ASR, analyte-specific reagents; PCR/CE, polymerase chain reaction/capillary electrophoresis; RFLP, restriction fragment length polymorphism.
Bold type indicates name of Assay.

UGT1A1 encodes UDP-glucuronosyltransferase, an enzyme responsible for the inactivation by glucuronidation of the active metabolite of irinotecan (SN38), a drug frequently used to treat patients with metastatic colorectal cancer. The FDA labeling of irinotecan suggests *UGT1A1* genotyping before treatment of the patient (*http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm215480.htm*, last accessed on June 15, 2010). The most common *UGT1A1* allele, *1, has 6 TA repeats in the TATA box of the promoter. In addition, there are three *UGT1A1* variant alleles that differ in the number of TA repeats (*28, *36 and *37 with 7, 5, and 8 TA repeats, respectively; Canada Research Chair in Pharmacogenetics *http://www.pharmacogenomics.pha.ulaval.ca/webdav/site/pharmacogenomics/shared/Nomenclature/UGT1A/UGT1A1.htm*, last accessed on March 10, 2010). These variants affect the expression and activity levels of the enzyme, and their allele frequency varies among different ethnic groups. Individuals homozygous for the *28 allele (10% of the U.S. population) metabolize irinotecan more slowly and are at increased risk for toxicity including high-grade neutropenia and/or diarrhea during irinotecan therapy (Pharmacogenomics Knowledge Base *http://www.pharmgkb.org/do/serve?objId=PA420&objCls=Gene*, last accessed on January 18, 2010).

This study describes the collaborative characterization of 107 publicly available genomic DNA reference materials for pharmacogenetic testing by the GeT-RM program and the genetic testing community.

Materials and Methods

Cell Line and Laboratory Selection

One hundred seven cell lines were selected from the National Institute of General Medical Sciences (NIGMS) Repository at the Coriell Cell Repositories for this study based on partial genotypic characterization, availability of DNA, and the varied ethnicities of the donors. All Coriell DNA and cell line materials are stripped of identifiers on submission and are assigned a Coriell cell line number. Therefore these samples are HIPPA compliant. Volunteer laboratories were selected based on assay platform as well as number of samples they were able to test. Both clinical genetic and commercial assay manufacturing laboratories participated in the study. DNA was prepared by Coriell, and sample sets were sent to the volunteer laboratories for genotyping. Participants used a variety of commercially available tests, both FDA-cleared and non–FDA-cleared, as well as laboratory developed tests (LDTs). Nearly all commercially available platforms were represented in this study (Table 1). The specific alleles detected by each assay varied (Table 1). Reagents for some of the assays were generously donated by Applied Biosystems Inc, AutoGenomics, Inc., Hologic, Inc. (formerly Third Wave Technologies), Luminex Molecular Diagnostics, Osmetech Molecular Diagnostics, and Roche Diagnostics.

DNA Preparation

Approximately 2 mg of DNA was prepared from each of the selected cell lines by the Coriell Cell Repositories using Gentra/Qiagen Autopure (Valencia, CA) per manufacturer's instructions or previously described methods.[8]

Assays Used in the Characterization Study

CYP2D6 *Assays*

AutoGenomics INFINITI platform. The AutoGenomics INFINITI 2D6 Mutation Detection Kit (AutoGenomics, Inc., Carlsbad, CA) simultaneously screens for 15 variants. Briefly, extracted genomic DNA was amplified by PCR, and alleles were distinguished using allele-specific primer extension (ASPE). Extension of primers resulted in incorporation of fluorescent CY5-dCTP. Extended primers were hybridized to complementary capture probes immobilized on the BioFilmChip and fluorescence was quantitated after scanning of the microarray using an integrated optics unit. Genotypes were assigned automatically by the proprietary software.[9]

Luminex xTag V2 platform. The Luminex xTag 2D6 Mutation Detection Kit (Luminex Molecular Diagnostics, Austin, TX) simultaneously screens for 15 variants. Briefly, genomic DNA was amplified in two PCR reactions, and alleles were discriminated using ASPE and hybridization to a universal microsphere array. Genotypes were detected on a Luminex 100 IS System and assigned using the proprietary TDAS software.[10]

ParagonDx. The ParagonDx 2D6 Real-time PCR reagents (ParagonDx, LLC, Morrisville, NC) simultaneously screen for nine alleles. Briefly, genomic DNA was amplified and singleplex alleles were discriminated using the Stratagene Mx3005P QPCR System platform. Genotypes were determined through end point fluorescence scatter plot analysis. *CYP2D6* *2A and *35 allelic genotypes were determined by cycle sequencing using BigDye Terminator Version 1.1 and the 3130xl Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA).[11]

Roche Amplichip platform. The Roche Amplichip P450 FDA-cleared Kit (Roche Diagnostics, Indianapolis , IN) simultaneously screens for 27 alleles in the *CYP2D6* gene. Genomic DNA was amplified in two multiplex PCR reactions. After fragmentation and labeling, the PCR products were denatured and hybridized to a microarray. The signal was detected on an Affymetrix GeneChip Scanner 3000Dx (Affymetrix, Santa Clara, CA) and alleles assigned by AmpliChip CYP450_US Data Analysis Software.[12]

The Applied Biosystems SNapShoT platform simultaneously screens for nine variants. Briefly, genomic DNA is amplified and extension primers of varying lengths are annealed to the amplicons. Each extension primer is specific for a single site and ends one base upstream from the mutation site. The primer extension reaction is performed using fluorescently-labeled dideoxynucleotides and the products are size-fractionated by capillary electrophoresis. Automated allele calling is accomplished using Genotyper software and associated macros (Applied Biosystems, Inc., Foster City, CA).[13]

CYP2C19 *Assays*

AutoGenomics INFINITI platform. The AutoGenomics INFINITI 2C19+ Assay (AutoGenomics, Inc., Carlsbad, CA) simultaneously screens for 10 variants. The procedure was as described above for *CYP2D6* using *CYP2C19* allele-specific PCR and detection primers and assay-specific microarrays.[14]

Luminex xTag V2 platform. The Luminex xTag 2C19 Mutation Detection Kit (Luminex Molecular Diagnostics, Austin, TX) simultaneously screens for seven variants. The procedure was as described above for *CYP2D6*.

Roche Amplichip platform. The Roche Amplichip P450 FDA-cleared Kit (Roche Diagnostics, Indianapolis , IN) simultaneously screens for two alleles in the *CYP2C19* gene. See assay description above.

Laboratory-developed test using restriction fragment length polymorphism (RFLP). This proprietary method for the detection of two variants includes the amplification of genomic DNA followed by restriction enzyme analysis. The restriction products are size-fractionated by agarose gel electrophoresis.

Laboratory-developed test for Taqman platform. Specimens were analyzed using the Applied Biosystems Taqman Drug Metabolism Genotyping Assays (Applied Biosystems, Inc., Foster City CA) for the detection of nine variants. This proprietary method includes the amplification of genomic DNA followed by dual-labeled oligonucleotides that hybridize to a specific target sequence. Hydrolysis by the 5′−3′ exonuclease activity of *Taq* polymerase releases the fluorescent reporter signal, permitting quantitative measurement of the accumulation of the PCR product via the fluorophore signal.[15] Manual allele calling is accomplished using ABI 7900HT version 2.3 Sequence Detection Systems (SDS) software.

CYP2C9, VKORC1 *assays*

AutoGenomics INFINITI platform. The AutoGenomics INFINITI CYP450 2C9-VKORC1 Assay (AutoGenomics, Inc., Carlsbad, CA) simultaneously screens for six variants in *CYP2C9* and eight variants in *VKORC1*. The procedure was as described above for *CYP2D6* using *CYP2C9* and *VKORC1* allele-specific PCR and detection primers and assay-specific microarrays.[9]

Idaho Technology, Inc. platform. The *CYP2C9* and *VKORC1* Genotyping Reagents [rapid melting curve analysis] (Idaho Technology, Inc., Salt Lake City Utah) simultaneously screens for 3 variants (*CYP2C9* *2 and *3, and *VKORC1* c.–1639G>A). Briefly, genomic DNA was amplified and alleles were discriminated using rapid melting curve analysis and simple probes.[16] Genotypes were assigned using derivative peak melting temperature analysis.[17,18]

Osmetech eSensor platform. The Osmetech eSensor Warfarin Sensitivity (IUO, Osmetech Molecular Diagnostics, Pasadena, CA) simultaneously screens for 10 variants

(*CYP2C9* *2, *3, *5, *6, *11, *14, *15, *16, *VKORC1* c.–1639 G>A, and *CYP4F2* c.1347 G>A). Briefly, genomic DNA was amplified and alleles were discriminated using a DNA microarray with electrochemical detection of hybridization in a microfluidic cartridge. Genotypes were assigned using the proprietary software.[9]

Laboratory-developed test using the Applied Biosystems SNapShot platform. This proprietary method for the detection of five variants was done as described above for *CYP2D6*.

Laboratory-developed test using the Luminex Eragen platform. Genomic DNA was amplified and alleles were discriminated using amplification primers and target-specific extension (TSE) probes (Eragen Biosciences, Madison WI). Genotypes were detected on a Luminex 100 or 200 IS Systems (Austin TX) and assigned using the Multicode-PLx Analysis Software (Eragen Biosciences, Madison WI).[19,20]

Laboratory-developed test for Taqman platform. Specimens were analyzed using the Applied Biosystems Taqman Drug Metabolism Genotyping Assays for the detection of eight variants as described above.

UGT1A1 *Assays*

AutoGenomics INFINITI platform. The AutoGenomics INFINITI *UGT1A1* Assay (AutoGenomics, Inc., Carlsbad, CA) simultaneously screens for 3 variants. The procedure was as described above for *CYP2D6* using *UGT1A1* allele-specific PCR, detection primers, and assay-specific microarrays.

Hologic, Inc. (formerly Third Wave Technologies) INVADER platform. The FDA-cleared UGT Kit (Hologic, Inc. Molecular Solutions, Bedford, MA) simultaneously screens for three variants; other alleles are not reported by the software. Invader probes hybridize to target DNA and a capture probe to create a single base, triple DNA strand structure that is cleaved to release an oligonucleotide flap. This oligonucleotide is captured by a universal fluorescence resonance energy transfer (FRET) cassette which is also cleaved at a single bp three-strand overlap to release a fluorescent signal. Subsequent rounds of flap oligo capture and FRET produces an amplified signal detection of the initial Invader SNP detection. The fluorescent signal is detected and genotypes are assigned using proprietary software. (Hologic Invader UGT1A1 Molecular Assay, Bedford, MA).

Laboratory-developed test using PCR and capillary electrophoresis. This proprietary method for the detection of three variants includes the amplification of genomic DNA followed by size-fractionation using capillary electrophoresis on an ABI3130xl and ABI3100 (Applied Biosystems, Inc., Foster City CA). Automated allele calling is accomplished using ABI software (Genemapper) and associated macros (Applied Biosystems, Inc., Foster City, CA).

Protocol

Each of the testing laboratories received one 10-μg aliquot of DNA from each of the cell lines to be tested. For logistical reasons (related to cost of reagents, staffing, and batched run size), the 107 DNA samples were divided into three nonoverlapping sets: Set 1 (35 DNA samples), Set 2 (36 DNA samples), and Set 3 (36 DNA samples). Based on an individual laboratory's resources and testing platform, each laboratory tested one or more sets of samples to ensure that each set was tested using multiple platforms. The samples were coded, and the expected genotypes were not revealed to the laboratories. DNA sample set(s) were analyzed using the laboratories' standard assay methods. The assay platforms used in the study, the alleles detected by each, and the sample set(s) tested with each method are indicated in Table 1. The results were submitted to the study coordinators (L.V.K. and V.M.P.), who examined the data for quality and discrepancies. If discrepancies were noted, the participating laboratory was requested to re-evaluate the sample in question (without providing the expected genotype) to determine whether there were analytical or postanalytical (such as transcription) errors. We did not perform direct DNA sequence analysis to resolve discrepancies because most of the participating laboratories were not able to provide this service.

Results

Each locus (*CYP2D6*, *CYP2C19*, *CYP2C9*, *VKORC1*, and *UGT1A1*) was tested in three to six laboratories using different assay platforms (Table 1). There were no false negative and two false positive results (described in detail below). The expected genotype (if known from previous data from Coriell) of each DNA sample was confirmed by all assay platforms designed to detect the alleles, and there was good overall concordance among laboratories. However, there were some differences in allele assignments related to the limitations of the manufacturer's assay design and variable nomenclature, especially for *CYP2D6*. The consensus genotype for each DNA sample is shown in Table 2. The alleles detected by each assay platform varied, but most of the common variants were identified among the set of 107 DNA samples. Table 3 lists the alleles that were interrogated for each locus as well as those that were identified or absent from the samples screened.

CYP2D6

CYP2D6 was the most challenging of the loci tested in this study. All of the assays used were designed to detect the *2 through *7, *10 and *17 alleles. Each of the assays detected various additional *CYP2D6* alleles, with the Roche Amplichip Assay detecting the largest number of alleles. Of the 26 possible *CYP2D6* alleles included in the assays used in this study, fifteen (*2, *3, *4, *5, *6, *9, *10, *17, *19, *29, *35, *41, *1XN, *2XN, and *4XN) were identified among the 107 DNA samples. Laboratories did not detect alleles *7, *11, *12, *14, *15, *19, *20, *36, *40,

Table 2. Consensus Genotypes[†]

Cell line number	Set 1: Consensus genotype				
	CYP2D6	*CYP2C19*	*CYP2C9*	*VKORC1* c.-1639G>A[‡]	*UGT1A1*
GM17227	*1/*9	*1/*1	*1/*2	GA	*28/*28
GM12244	*35/*41	*1/*1	*2/*3	GG	*1/*28
GM17240	*1/*10	*1/*1 (*1/*17)	*1/*1	AA	*1/*1
GM17289	*2/*4	*2/*2	*1/*1	AA	*1/*1
GM17221	*1XN/*2	*1/*1	*2/*3	GA	*1/*1
GM17119	*1/*2	*1/*1 (*1/*17)	*1/*1	GG	*1/*37
GM17129	*1/*4	*1/*1	*1/*2	GG	*1/*36
GM17235	*1/*5	*1/*1	*1/*1	GG	*1/*28
GM17203	*4/*35	*1/*2 (*2/*17)	*1/*1	GA	*1/*1
GM17058	*10/*10	*1/*2	*1/*1	AA	*1/*28
GM17281	*5/*9	*1/*1 (*1/*17)	*1/*1	GA	*1/*28
GM17293	*2/*9	*1/*1 (*1/*17)	*1/*2	GA	*28/*28
GM17115	*1/*2	*1/*1	*1/*1 (*9/*9)	GG	*28/*28
GM17114	*1/*5	*1/*1	*1/*1	GG	*1/*28
GM17084	*1/*10	*2/*4	*1/*2	GA	*1/*1
GM17039	*2/*17	*1/*1	*1/*1 (*1/*9)	GG	*1/*36
GM17300	*1/*6	*1/*1 (*1/*17)	*1/*1	GA	*1/*28
GM17210	*1/*4	*1/*1	*1/*2	AA	*1/*1
GM17247	*1/*2	*1/*1	*3/*3	GA	*1/*28
GM10005	*17/*29	*1/*1 (*1/*17)	*1/*1 (*1/*9)	GG	*1/*1
GM17057	*1/*10	*1/*1	*1/*1	GA	*1/*1
GM17280	*2/*3	*1/*8	*1/*2	GG	*1/*1
GM17252	*4/*5	*1/*1	*2/*3	GA	*28/*28
GM17204	*1/*35	*1/*1	*1/*3	AA	*1/*1
GM09301	Duplication	*1/*1 (*1/*17)	*1/*1	GG	*28/*28
GM02016	*2XN/*17	*1/*2	*1/*1	GA	*1/*1
GM17272	*4/*10	*1/*1 (*17/*17)	*1/*1	AA	*1/*28
GM17296	*1/*9	*1/*1 (*17/*17)	*1/*1	GA	*28/*28
GM17298	*1/*1XN	*1/*1	*1/*1	GA	*1/*28
GM17248	*4/*10	*1/*1 (*17/*17)	*1/*1	AA	*28/*28
GM12273	*1/*1	*1/*2	*1/*2	GG	*1/*28
GM17246	*4/*35	*1/*8 (*8/*17)	*1/*2	GA	*1/*28
GM07439	*4XN/*41	*2/*2 (*2/*10)	*1/*1 (*1/*9)	GG	*1/*28
GM17130	*1/*2	*1/*1 (*1/*17)	*1/*3	GG	*1/*37
GM17052	*1/*1	*1/*3	*1/*1	AA	*1/*1

Cell line number	Set 2: Consensus genotype				
	CYP2D6	*CYP2C19*	*CYP2C9*	*VKORC1* c.-1639G>A[‡]	*UGT1A1*
GM17215	*4/*41	*1/*1	*1/*3	GG	*1/*1
GM16688	*2/*10	*2/*3	*1/*1	AA	*1/*1
GM17245	*2/*4	*1/*2	*1/*2	GA	*1/*28
GM17218	*2/*2 (*35)	*1/*2	*1/*1	GG	*1/*1
GM17438	*2/*4	*1/*1	*1/*2	AA	*28/*37
GM17618	*1/*2	*1/*1	*1/*1	GA	*1/*28
GM17213	*1/*2 (*35)	*1/*1 (*1/*17)	*1/*1	GA	*1/*1
GM17285	*1/*1	*1/*1	*2/*3	AA	*1/*1
GM17209	*1/*4	*1/*1	*1/*2	GG	*1/*28
GM17216	*1/*1	*1/*1	*1/*2	AA	*1/*28
GM17208	*2/*41	*1/*1	*1/*1	GA	*28/*28
GM17237	*1/*4	*1/*2 (*2/*17)	*1/*1	AA	*1/*28
GM17232	*2/*2XN	*1/*2 (*2/*17)	*1/*1	GA	*1/*1
GM17230	*4/*41	*1/*1 (*1/*17)	*1/*1	GA	*1/*1
GM17207	*2/*41	*1/*2	*1/*1	GG	*28/*28
GM17260	*2/*2	*1/*2 (*2/*17)	*1/*1	GA	*1/*28
GM17242	*1/*1	*1/*1	*1/*1	GA	*1/*28
GM17254	*4/*41	*1/*1	*2/*3	GG	*1/*28
GM17256	*2 (*35)/*2 (*35)	*1/*1	*1/*1	GA	*1/*1
GM17201	*1/*1	*1/*2	*1/*1	GA	*1/*28
GM16689	*2/*10	*2/*2	*1/*1	AA	*1/*1
GM17205	*1/*41	*1/*2	*1/*2	GA	*1/*1
GM17078	*1/*1	*1/*2	*1/*2	GG	*1/*36
GM17440	*1/*1	*1/*1	*1/*1	GA	*1/*28
GM17466	*1/*2	*1/*1 (*1/*17)	*1/*1	GG	*1/*1
GM17277	*1/*1	*1/*1 (*1/*17)	*1/*1	AA	*1/*28

(*table continues*)

Table 2. *Continued*

Cell line number	Set 2: Consensus genotype				
	CYP2D6	*CYP2C19*	*CYP2C9*	*VKORC1* c.-1639G>A‡	*UGT1A1*
GM17020	*1/*10	*1/*1	*1/*1	AA	*1/*1
GM17220	*1/*4	*1/*1 (*1/*17)	*1/*1	GA	*1/*28
GM17228	*1/*1	*1/*1	*1/*2	GG	*1/*28
GM17224	*1/*1	*1/*2	*1/*1	GG	*1/*1
GM17206	*2/*4	*1/*1	*1/*3	GG	*1/*28
GM17211	*2/*4	*1/*1	*1/*3	GA	*1/*1
GM17226	*4/*4	*1/*1	*1/*2	GA	*28/*28
GM17219	*1/*1	*1/*2	*1/*1	GG	*28/*28
GM17262	*2/*41	*1/*1 (*17/*17)	*1/*1	GG	*1/*1
GM17222	*1/*2	*1/*1	*2/*2	GA	*28/*28

Cell line number	Set 3: Consensus genotype				
	CYP2D6	*CYP2C19*	*CYP2C9*	*VKORC1* c.-1639G>A‡	*UGT1A1*
GM17214	*2/*2	*1/*2	*1/*1	GG	*1/*1
GM17244	DUP/*4/*2A	*1/*1	*1/*1	AA	*1/*1
GM17217	*1/*41	*1/*1	*1/*1	GG	*1/*28
GM17073	*1/*17	*1/*1	*1/*1	GG	*28/*28
GM17282	*41/*41	*1/*1	*1/*1	GG	*28/*28
GM17295	*1/*4	*1/*2	*1/*2	GA	*1/*28
GM17233	*1/*41	*1/*1	*1/*1	GG	*1/*1
GM17269	*2/*41	*1/*2	*1/*1	GA	*1/*1
GM17264	*1/*4	*1/*2 (*2/*17)	*1/*1	GG	*1/*1
GM17265	*2/*2	*1/*1	*1/*1	AA	*1/*28
GM17292	*4/*4	*1/*2 (*2/*17)	*1/*1	GA	*1/*28
GM17288	*1/*1	*1/*2 (*2/*17)	*1/*1	GA	*1/*28
GM17287	*1/*1 (*36/?)	*1/*1 (*1/*17)	*1/*1	GG	*1/*28
GM17257	*2/*4	*1/*1 (*1/*17)	*1/*1	GA	*28/*28
GM17019	*1/*10	*1/*1	*1/*3	GA	*1/*1
GM17290	*1/*41	*1/*2	*1/*3	GG	*1/*1
GM17276	*2/*5	*1/*1 (*1/*17)	*1/*1	GG	*1/*28
GM17268	*1/*4	*1/*1	*1/*2	GG	*1/*1
GM17291	*1/*4	*1/*1	*1/*3	GA	*1/*28
GM17283	*4/*41	*1/*1	*1/*2	GG	*1/*1
GM17243	*2 (*35)/*4	*1/*1 (*1/*17)	*1/*2	AA	*1/*1
GM17249	*1/*41	*1/*1 (*1/*17)	*1/*2	GA	*1/*1
GM17275	*1/*1	*1/*1 (*1/*17)	*1/*1	GA	*1/*28
GM17234	*1/*41	*1/*1	*1/*3	GG	*1/*1
GM16654	*10/*10	*1/*2	*1/*1	GA	*1/*1
GM17212	*1/*1	*1/*2	*1/*2	GG	*1/*28
GM17229	*1/*2	*1/*1	*1/*1	AA	*1/*28
GM17261	2 (*35)/*4	*1/*1	*1/*2	GA	*1/*28
GM17286	*1/*4	*1/*1	*1/*1	GA	*1/*28
GM17236	*2/*41	*1/*1	*1/*1	GG	*1/*28
GM17267	*1/*4	*1/*1	*1/*1	GA	*1/*1
GM17231	*1/*2	*1/*1	*1/*2	GG	*1/*28
GM17075	*1/*2	*1/*1	*2/*2	GA	*1/*28
GM17263	*1/*1	*2/*2	*1/*1	GA	*1/*28
GM17274	*1/*2	*1/*1 (*1/*17)	*1/*2	GA	*1/*1
GM17279	*4/*41	*1/*1 (*17/*17)	*1/*1	GG	*1/*1

† Genotypes in parentheses were detected by assays performed in only one laboratory. The results were not verified because no other assays designed to detect the allele were included in this study.

‡ *VKORC1*, only c.-1639G>A (3673) is indicated. Data for other alleles are available on the GeT-RM Web site *http://wwwn.cdc.gov/dls/genetics/rmmaterials/default.aspx.*

*10XN, *17XN, *35XN, and *41XN in any of the samples tested.

Several discrepancies were identified in the *CYP2D6* data (Table 4). We identified one false positive result for GM17289. This assay was not repeated, so we were unable to resolve the cause of the false positive result (*29, Autogenomics), however, the results from the other platforms agreed with one another, therefore this sample was assigned the genotype *2/*4. Additionally, one platform, the Roche Amplichip, was unable to genotype several samples (GM17084, GM17252, GM17244, and GM17287) due to limitations of the allele-calling algorithm used for interpretation in this assay format (Table 4). These "no calls" were likely due either to a combination of detected alleles which was not recognized by the software or to the presence of some rare alleles that were detected by the AmpliChip CYP450 Test, but were not reported because their phenotypes were not known.

Table 3. Alleles Identified in the DNA Samples Tested

	Locus				
	CYP2D6	*CYP2C19*	*CYP2C9*	*VKORC1*†	*UGT1A1*
Alleles found in study samples	*2, *3, *4, *5, *6, *9, *10, *17, *19, *29, *35, *41, *1XN, *2XN, *4XN	*2, *3, *4, *8, *10, *17	*2, *3, *9	3673 (c.-1639G>A), 5808, 6009, 6484, 6853, 7566, 8773, 9041	*28, *36, *37
Alleles not found in study samples	*7, *11, *12, *14, *15, *19, *20, *36, *40, *10XN, *17XN, *35XN, *41XN	*5, *6, *7, *9, *12	*4, *5, *6, *8, *11, *12, *14, *15, *16		
Alleles interrogated by only one method	*19, *20, *36, *40, *35XN	*10, *12, *17	*4, *8, *9, *12, *14, *15, *16	5808, 6009, 6484, 6853, 7566, 8773, 9041	

† VKORC1, only c.-1639G>A (3673) is indicated. Data on the other alleles available on the GeT-RM Web site (*http://wwwn.cdc.gov/dls/genetics/rmmaterials/default.aspx*.

Alleles not tested in sets 2 and 3 are underlined.

When discrepant results among assays were identified, the SNPs detected by each assay were examined to determine the consensus *alleles in each sample, reported in Table 2. There were also several *CYP2D6* gene duplications identified. Some of the platforms/assays do not specify which allele is duplicated and in one sample (GM09301) a consensus genotype was not obtained (Table 4).

The *CYP2D6* *2B allele (c.1039C>T, c.1661G>C, c.2850C>T, c.4180G>C) was recently reclassified as *35 (c.-1584C>G, c.31G>A, c.1661G>C, c.2850C>T, c.4180G>C). However, functionally they are equivalent. This change affected six samples (GM17218, GM17213, GM17256, GM17287, GM17243, and GM17261). All six were identified by the FDA-cleared Roche Amplichip with at least one *35 allele. The two other platforms (AutoGenomics INFINITI and Luminex xTag) do not discriminate the *35 allele (and genotyped these samples as *CYP2D6* *2) and therefore *35 could not be verified by these assays for these samples (Table 4).

The *CYP2D6* genotype of sample GM17287 was designated a "No Call" by the Roche Amplichip (Table 4). On examination of the SNPs detected, the sample was positive for *36, however the Roche assay was the only one designed to detect *36 which is described as a gene conversion in exon 9 (*www.cypalleles.ki.se/cyp2d6.htm*, last accessed on June 14, 2010), thus a CYP2D6 consensus genotype of *1/*1 was assigned because the *36 was not confirmed by any of the other assays.

Finally, the results for sample GM17119 were also discrepant. On examination of the algorithms used by each assay to determine allele status, this sample was assigned a *CYP2D6* consensus genotype of *1/*2 rather than *1/*41 (genotype obtained by Roche Amplichip), because it did not have the *41 defining

Table 4. Discrepant CYP2D6 Genotypes Listed by Platform†

Coriell cell line number	Consensus result‡	Roche Amplichip®	LDT	AutoGenomics INFINITI®	Luminex XTag™ V2	ParagonDx§
GM17289	*2/*4	*2/*4	*2/*4	*2 (*29)/*4,*10	*2A/*4	*2A/*4
GM17084	*1/*10	NC¶	*2/*10	*2/*10	*1/*10	*1/*10
GM17252	*4/*5	NC‖	*4/*5	*4,*10/*5,	*4/*5	*4/*5
GM17244	DUP/*4/*2A	NC**	NA¶	*2/*4,*10, XN	DUP/*4/*2A	NA
GM17287	*1/*1 (*36/?)	NC††	NA	*1/*1	*1/*1	NA
GM09301	Duplication	*1/*41XN	*2/*X2 (DUP)	*2/XN	DUP/*2	*1/*1XN
GM17218	*2/*2 (*35)	*2/*35	NA	*2/*2	*2A/*2A	NA
GM17213	*1/*2 (*35)	*1/*35	NA	*1/*2	*1/*2A	NA
GM17256	*2 (*35)/*2 (*35)	*35/*35	NA	*2/*2	*2A/*2A	NA
GM17243	*2 (*35)/*4	*4/*35	NA	*2/*4,*10	*2A/*4	NA
GM17261	*2 (*35)/*4	*4/*35	NA	*2/*4,*10	*2A/*4	NA
GM17119	*1/*2	*1/*41	*1/*2	*1/*2	*1/*2	*1/*1

† Where no mutation is detected, *1 is assumed.

‡ Genotypes in parentheses were detected by assays performed in only one laboratory. The results were not verified because no other assays designed to detect the allele were included in this study.

§ NA, not applicable, as platform was not used to test DNA from the cell line.

¶ NC, No call (*10; Haplotype: c.100C>T, c.1039C>T, c.1661G>C, c.2850C>T, c.4180G>C).

‖ NC, No call [*4/*5; Haplotype: c.100C>T(mut), c.1039C>T, c.1661G>C(mut), c.1846G>A(mut), c.4180G>C(mut); deletion detected].

** NC, No call [*2/*4 duplication identified; Haplotype: c.-1584C>G, c.100C>T, c.1661G>G(mut), c.1846G>A, c.2850C>T, c.4180G>C(mut); duplication positive].

†† NC, No call (*36/?; Haplotype: *36 gene conversion, c.4180G>C).

c.2988G>A SNP based on negative findings by AutoGenomics INFINTI, Luminex xTag and ParagonDx assays that are designed to detect this specific SNP (Tables 1 and 4).

CYP2C19

The *CYP2C19* assays used in this study are designed to detect a variety of *CYP2C19* polymorphisms, including the common nonfunctional (poor metabolizing) variants *2 and *3. Several of the assays have the capacity to detect the nonfunctional *4, *5, *6, *7, and *8 alleles, and a few of these are also capable of detecting one or more of the decreased function *9, *10, and *12 alleles as well as the increased function *17 allele (Table 1) (Human Cytochrome P450 [CYP] Allele Nomenclature Committee *http://www.cypalleles.ki.se/cyp2c19.htm*, last accessed on February 11, 2010). This study identified genomic DNA samples containing *2, *3, *4, *8, *10 and *17 (Table 2). We did not detect several other *CYP2C19* alleles included in the assays used (Table 3). The Luminex assay reported a *1/*2 genotype for sample GM07439. This assay was not repeated by the laboratory to confirm the result. The other platforms, however, reported that this sample was either a *2/*2 or a *2/*10, so we assigned it a *2/*2 (*2/*10) genotype. Only one of the assays used is designed to detect the *10 allele, which shares SNPs with the *2 allele, thus its presence in this sample has not been confirmed.

CYP2C9, VKORC1

All seven laboratory tests identified the decreased function alleles *CYP2C9* *2, *3 (*1 was assumed when no mutations were detected), and *VKORC1* c.−1639 (3673) G>A among the 107 DNA samples of the study set (Table 2). These are the most common variant alleles observed in the white population, which is the ethnicity represented by >75% of samples in the study. In addition, one of the assays detected *CYP2C9* *9, an allele specific to blacks.[21] Only one laboratory tested for this variant and reported that three cell lines were heterozygous for the *9 allele and one was homozygous for this variant. Currently this finding has not been confirmed using any other methods. Additional *CYP2C9* alleles were included in some of the assays but were not detected among the 107 samples, although sample sets 2 and 3 were not screened with methods that detected other variant alleles (Tables 1 and 3). The AutoGenomics platform is designed to detect *VKORC1* c.−1639 (3673) G>A as well as seven other *VKORC1* alleles that are not included in the other assays. All of the alleles included in this platform were identified in one or more of the 107 samples tested (Table 3, data available on the GeT-RM website*http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx*, last accessed on September 16, 2010). There was complete concordance among laboratories for each of the *CYP2C9* and *VKORC1* variant alleles within the detection capacities of the test methods used.

UGT1A1

All of the *UGT1A1* assay platforms used in this study were designed to detect three *UGT1A1* variant alleles (*28, *36 and *37; *1 was assumed if no variant allele is detected). We were able to identify each of these alleles in one or more of the107 DNA samples (Table 3). There was complete concordance among laboratories for each of the *UGT1A1* variant alleles within the detection capacities of the test methods used.

Other Loci

A subset of laboratories also reported results for 15 other pharmacogenetic (*CYP4F2*, *EPHX1*, *ABCB1*, *HLAB*, *KIF6*, *CYP3A4*, *CYP3A5*, *TPMT*, *DPD*) and nonpharmacogenetic loci (*F5*, *F2*, *HFE*, *MTHFR*, *AAT*, *PAI1*) (data available on the GeT-RM website *http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx*, last accessed on September 16, 2010). Each of these additional loci was characterized by one or two laboratories depending on the assays available in the laboratory and the capacity to assay the samples.

Discussion

This study describes the characterization of 107 publicly available cell line–derived genomic DNA samples for five pharmacogenetic loci, *CYP2D6*, *CYP2C9*, *VKORC1*, *CYP2C19,* and *UGT1A1* that are commonly included in clinical pharmacogenetic testing. Each sample was tested in multiple laboratories using a variety of assay platforms, although there are a number of other platforms that were not included in this study. This strategy ensures that the DNA would be thoroughly characterized and will be commutable among a variety of assay platforms. We were able to identify many, but not all, of the alleles commonly included in clinical assays for the five loci. The GeT-RM program will further this work with the pharmacogenetic testing community and the Coriell Cell Repositories to identify cell lines with these missing alleles and undertake the necessary characterization studies. In addition to the five loci characterized by multiple laboratories, this study also generated genotypic information for an additional fifteen pharmacogenetic and nonpharmacogenetic loci. These data will be useful to laboratories developing and validating new genetic tests. The GeT-RM will use the initial genotypic results from these additional 15 loci as a starting point for future reference material characterization projects. This may include genotyping DNA from additional cell lines to identify samples that encompass all of the alleles in available platforms. Also the 107 cell lines' DNA may be characterized for additional PGx markers or the initial genotype results may be confirmed using a multilaboratory study.

Currently there are a number of commercially available platforms as well as LDTs used for clinical testing of PGx loci. With the exception of a few commercially available cell lines and synthetic DNA products, some of which are FDA-cleared and some of which are Research or Investigational

Use Only, characterized genomic DNA RMs are not available for PGx testing. International, federal, and state regulations and professional guidelines require the inclusion of reference or quality control materials during the analysis of patient samples[22–26] (European Molecular Genetics Quality Network, *http://emqn.org/eqmn/bestpractice.html*, last accessed on August 4, 2009; Washington State Legislature, *http://apps.leg.wa.gov/WAC/default.aspx?cite_246–338*, last accessed on August 4, 2009; New York State Clinical Laboratory Evaluation Program, *http://www.wadsworth.org/labcert/clep/clep.html*, last accessed on August 4, 2009; College of American Pathologists, *http://www.cap.org*, last accessed on August 4, 2009; American College of Medical Genetics, *http://www.acmg.net/Pages/ACMG_Activities/stds-2002/g.htm*, last accessed on August 4, 2009). Reference materials are also used for development of new genetic tests including those for PGx, test validation, and other quality assurance purposes.[23]

In the absence of available characterized reference materials, laboratories often use unconfirmed and nonrenewable sources of genomic DNA such as residual patient specimens. This approach, while feasible, is not optimal because samples with uncommon alleles are often difficult to obtain and the supply of these materials is limited. In addition, the residual samples used as reference materials should be characterized using a method other than the one used to test them initially, or by an independent laboratory.[27] The public availability of a renewable source of genomic DNA reference materials that have been characterized using a variety of methods, such as the 107 genomic DNA reference materials characterized in this study, will also facilitate assay standardization among laboratories. It should be noted that the purified genomic DNA materials characterized in this study serve only as positive controls for analysis of PGx polymorphisms. It does not control for the entire testing process, including DNA extraction from whole blood.

The Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee website (*http://www.cypalleles.ki.se/* , last accessed on January 7, 2010) indicates the defining sequence variants for the major *alleles of each of the *CYP* genes. Allele nomenclature may change over time and is updated as more information becomes available regarding the functional SNPs and enzymatic activity of each allele. The SNPs used to define each of the *CYP2C9*, *CYP2C19*, *VKORC1*, and *UGT1A1* *alleles were consistent among the assays used in this study (Table 5). However, the SNPs which define the *CYP2D6* *alleles were highly variable between assays. Some assays/platforms only detect the major SNP that defines each of the *CYP2D6* *alleles (AutoGenomics INFINITI, ParagonDX, and a LDT in our study), whereas other assays/platforms detect multiple SNPs for each *CYP2D6* *allele (Luminex xMap and Roche Amplichip).

Variability in allele definition between various assay platforms can create discrepant results and reporting. There are two examples of this within the *CYP2D6* data set: *4 versus *10 and *2 versus *41 (Table 4). *CYP2D6* *4 and *10 enzymes, have no and decreased activity, respectively. The major SNP in *4 is c.1846G>A, which causes a splicing defect that abrogates enzymatic activity, but other SNPs in the *4 haplotype may include the following: c.100C>T, c.974C>A, c.984A>G, c.4180G>C. In *10, the major defining SNP is c.100C>T, which causes a proline to serine change associated with decreased enzymatic activity. Both *4 and *10 alleles include c.100C>T and c.4180G>C, which is also contained in *2 and other *CYP2D6* alleles, indicating that many of the variant alleles likely arose on the same haplotype background (Table 5) (Human Cytochrome P450 [CYP] Allele Nomenclature Committee *http://www.cypalleles.ki.se/cyp2d6.htm*, last accessed on February 11, 2010). Presence of c.100C>T and c.1846G>A is consistent with a *4 allele, but the Autogenomics assay reports both *10 and *4 for this SNP combination. The potential exists that a *1/*4 patient could be misidentified as *4/*10 due to the inability to assign the correct allele based on the combination of SNPs present. This could affect clinical management of drug therapy because *4/*10 (nonfunctional allele/decreased functional allele) and *1/*4 (normal functional allele/nonfunctional allele) have a different predicted phenotype. In the Roche package insert, *1/*4 is assigned an extensive metabolizer phenotype, while *4/*10 is assigned an intermediate metabolizer phenotype. How laboratory directors using SNP-only platforms are assigning genotypes and metabolizer status is not part of this project but remains a potential concern in clinical testing. Additionally, assigning phase to the various SNPs is not addressed by this study but may have implications in result interpretation and thus deserves further review.

The other major discrepancy in *CYP2D6* genotyping was differentiating *2 from *41. The *2 allele is defined by c.2850C>T and c.4180G>C and is associated with normal enzymatic function. The defining SNP in *41 is c.2988G>A, which is associated with decreased enzymatic activity due to altered RNA splicing.[28,29] The *41 haplotype also includes c.2850C>T, c.4180G>C, and c.-1584C, which are also reported in some subsets of *2. The Roche Amplichip does not include the defining *41 SNP c.2988G>A and thus may misclassify some *2 alleles (*2L and *2M) as *41. This confusion arose because it was unclear from initial studies which single *41 SNP was responsible for decreased enzymatic function. Once studies showed an association between c.2988G>A and decreased enzymatic activity, alleles with c.-1584C but not c.2988G>A were reclassified as subsets of *2 (normal enzymatic activity). This affected results for sample GM17119, which varied among platforms (Table 4).

In addition, there were other changes in allele nomenclature that had an impact on the consensus genotypes reported for DNAs characterized in this study. The Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee reclassified *2B as *35 (c.-1584C>G, c.31G>A, c.1661G>C, c.2850C>T, c.4180G>C), and the SNPs c.1039C>T, c.1661G>C, c.2850C>T, c.4180G>C to define *2B. This nomenclature change does not have phenotype interpretation consequences, because both are functional alleles. In our study, this change impacted six samples (GM17218, GM17213, GM17256, GM17287, GM17243, and GM17261) (Table 4). All six of these DNA

Table 5. Defining SNPs for Major Alleles of *CYP2D6*, *CYP2C9*, *VKORC1*, *CYP2C19*, and *UGT1A1*

SNP	Allele[†]	dbSNP rs#
*CYP2D6**2	c.2850C>T, c.4180G>C	rs16947,
*CYP2D6**3	**c.2549delA**	rs4986774
*CYP2D6**4	c.100C>T, **c.1846G>A**, c.4180G>C	rs3892097
*CYP2D6**5	**Whole gene deletion**	
*CYP2D6**6	**c.1707delT**	rs5030655
*CYP2D6**7	**c.2935A>C**	
*CYP2D6**8	**c.1758G>T**, c.2850C>T, c.4180G>C	
*CYP2D6**9	**c.2615_2617delAAG**	rs28371720
*CYP2D6**10	**c.100C>T**, c.4180G>C	rs1065852
*CYP2D6**11	**c. 883G>C**, c. 2850C>T, c.4180G>C	
*CYP2D6**12	**c.124G>A**, c.2850C>T, c.4180G>C	
*CYP2D6**14	**c.1758G>A**, c.2850C>T, c.4180G>C	
*CYP2D6**15	**c.138insT**	
*CYP2D6**17	**c.1023C>T**, c.2850C>T, c.4180G>C	rs28371706
*CYP2D6**19	**c.2539–2542delAACT**, c.2850C>T, c.4180G>C	
*CYP2D6**20	**c.1973insG**, c.2850C>T, c.4180G>C	
*CYP2D6**29	c.2850C>T, c.3183G>A, c.4180G>C	
*CYP2D6**35	c.31G>A, c.2850C>T, c.4180G>C	
*CYP2D6**36	c.100C>T, c.4180G>C, **gene conversion in exon 9**	
*CYP2D6**40	c.1023C>T, **c.1863ins(TTTCGCCCC)2**, c.2850C>T, c.4180G>C	
*CYP2D6**41	c.2850C>T, **c.2988G>A**, c.4180G>C	rs28371725
*CYP2C9**2	c.430C>T	rs1799853
*CYP2C9**3	c.1075A>C	rs1057910
*CYP2C9**4	c.1076T>C	
*CYP2C9**5	c.1080C>G	rs28371686
*CYP2C9**6	c.818delA	rs9332131
*CYP2C9**8	c.449G>A	rs7900194
*CYP2C9**9	c.752A>G	rs2256871
*CYP2C9**11	c.1003C>T	rs28371685
*CYP2C9**12	c.1465C>T	rs9332239
*CYP2C9**14	c.374G>A	
*CYP2C9**15	c.485C>A	
*CYP2C9**16	c.895A>G	
*CYP2C19**2	c.681G>A	rs4244285
*CYP2C19**3	c.636G>A	rs4986893
*CYP2C19**4	c.1A>G	rs28399504
*CYP2C19**5	c.1297C>T	rs56337013
*CYP2C19**6	c.395G>A	
*CYP2C19**7	g.19294T>A	
*CYP2C19**8	c.358T>C	rs41291556
*CYP2C19**9	c.431G>A	rs17884712
*CYP2C19**10	c.680C>T	
*CYP2C19**12	c.1473A>C	rs55640102
*CYP2C19**17	c.99C>T, c.991A>G	
VKORC1	c.-1639G>A	rs9923231
*UGT1A1**28	A $(TA)_7$TAA	rs81753476
*UGT1A1**36	A $(TA)_5$TAA	rs81753476
*UGT1A1**37	A $(TA)_8$TAA	rs81753476

[†] Defining SNP in bold.

samples were identified by the FDA-approved Roche Amplichip as having at least one *35 allele. The two other platforms (AutoGenomics INFINITI and Luminex *xTag*) are not designed to identify the *35 allele because they do not detect c.31G>A; thus this genotype could not be confirmed. Luminex *xTag* detects only *2A, while the AutoGenomics INFINITI system does not indicate which *2 subtype it detects. Changes to allele assignments are made as more information is available; however, new information resulting in nomenclature changes can impact both manufacturers and laboratory scientists developing clinical assays and adds confusion to interpretation of clinical results.

In conclusion, we have characterized a set of 107 genomic DNA RMs for five pharmacogenetic loci (*CYP2D6*, *CYP2C19*, *CYP2C9*, *VKORC1*, and *UGT1A1*), using a variety of assay platforms in multiple pharmacogenetic testing laboratories. These samples can be used for quality assurance, proficiency testing, and test development and research and should help ensure the accuracy of pharmacogenetic testing. These pharmacogenetic RMs, as well as other materials developed by GeT-RM, are publicly available from the NIGMS repository at the Coriell Cell Repositories (*http://ccr.coriell.org/Sections/Collections/NIGMS/?SsId=8*, last accessed on April 13, 2010). More information on this and other reference material characterization projects is available at the GeT-RM website: *http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx*, last accessed on September 16, 2010.

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