Coverage of the PharmaADME Consortium Core Marker List by TaqMan® DME and Copy Number Assays

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ABSTRACT

The PharmaADME Consortium [1] is composed of individuals from academia and pharmaceutical and genomic technology industries, who together developed a consensus list of key genes involved in the absorption, distribution, metabolism and excretion (ADME) of drugs. The list includes phase 1 and 2 drug metabolizing enzymes and transporter genes. A core list of 184 putative functional genetic variants in 33 significant ADME genes, which likely impact the pharmacokinetics of drug metabolism, was also created. By providing this information to technology providers, products could be developed to support pharmacogenetic research, with the objective of improving the quality of pharmaceuticals available to consumers. We report here on our progress towards covering the core ADME marker list with TaqMan® Genotyping Assays.

INTRODUCTION

The ADME marker list includes 172 SNP and insertion/deletion (InDel) polymorphisms, 10 copy number variants (CNV), 1 hybrid gene, and 1 microsatellite polymorphism. We first reviewed our available list of TaqMan® Drug Metabolism (DME) Genotyping Assays, which detect polymorphisms in coding and regulatory regions in 221 DME genes. We then focused on developing allelic discrimination assays to any SNP or InDel ADME marker for which we did not have an assay. Challenges for assay design included: assay specificity for targets with high homology to pseudogenes or gene family members, triallelic SNP targets, other SNPs in close proximity to the target, and repetitive target context sequences. For some challenging targets, several rounds of assay design and testing were required to obtain a robust TaqMan® DME Assay. Here we report that 164 DME assays covering SNP and InDel ADME targets are now, or will soon be, available. Efforts are underway to develop assays for the remaining targets. TaqMan® Copy Number (CN) Assays were developed for the core list 10 copy number variants (CNV) and the hydrid gene, which encompass deletions, duplications, and gene conversions in 6 total genes. In total, over 95% of the core ADME markers are covered by TaqMan® DME or CN Assays, which offer a simple, accurate and scalable workflow for genotyping these important alleles in pharmacogenetic research.

RESULTS

Assay Designs were primarily generated using AB proprietary assay design pipelines. Manual adjustments to designs were sometimes made to improve assay performance. DME assays contain allele-specific FAM™ and VIC® dye MGB probes and a primer pair. CN assays contain a FAM™ dye MGB probe and a primer pair.

TaqMan® DME Genotyping Assays were tested on 4 population panels of 45 samples each (African American and Caucasian Coriell gDNAs [2]; Chinese and Japanese collaborator gDNAs). 5 µl reactions contain 3 or 1 ng DNA, assay, and TaqMan® Universal Master. PCR was performed on an AB 9700 thermocycler using DME assay cycling conditions: 10 mins at 95°C followed by 50 cycles: 92° C 15 sec, 60° C 90 sec. Plates were read on the AB 7900HT Fast Real-Time PCR System using the SDS v2.3 software Allelic Discrimination end point read program. Results export files were uploaded into AutoCaller™ Software for data analysis.

TaqMan® Copy Number Assays were tested with at least the African American and Caucasian population panels (see above). CN assays were run together with the RNase P H1 RNA reference assay (VIC® dye-TAMRA™ probe), 10 ng gDNA and TaqMan® Genotyping Master Mix in a 10 µl duplex PCR (4 replicates per sample). PCR reactions were run on an AB 7900HT System, using Absolute Quantitation settings, for 10 mins at 95°C followed by 40 cycles: 95°C 15 sec, 60°C 60 sec. Real-time PCR results were analyzed by SDS v2.3 software using autobaseline and manual C_t threshold of 0.2. Results export files were opened in CopyCaller™ Software for sample copy number analysis by the relative quantitation method.

RESULTS

Table 1. Coverage of PharmaADME Core SNP and InDel Markers by TaqMan® DME Assays

Category	Gene symbol	# of core markers	# DME assays
transporter	ABCB1	5	5
transporter	ABCC2	6	6
transporter	ABCG2	2	2
phase I	CYP1A1	9	9
phase I	CYP1A2	4	4
phase I	CYP2A6	9	8
phase I	CYP2B6	4	4
phase I	CYP2C19	9	9
phase I	CYP2C8	7	7
phase I	CYP2C9	14	13
phase I	CYP2D6	24	22
phase I	CYP2E1	1	1
phase I	CYP3A4	3	2
phase I	CYP3A5	5	5
phase I	DPYD	6	6
phase II	GSTM1	1	1
phase II	GSTP1	1	1
phase II	NAT1	7	7
phase II	NAT2	8	8
transporter	SLC15A2	4	4
transporter	SLC22A1	10	9
transporter	SLC22A2	5	5
transporter	SLC22A6	1	1
transporter	SLCO1B1	10	10
transporter	SLCO2B1	1	1
phase II	SULT1A1	3	0
phase II	TPMT	6	6
phase II	UGT1A1	5	5
phase II	UGT2B15	1	1
phase II	UGT2B7	2	2
Total	33 genes	172	164

The 33 core PharmaADME genes and their functional category are listed. The number of core SNP and small InDel markers for each gene, and coverage by TaqMan® DME Assays, are indicated.

▪ 2 markers are haplotypes containing SNP targets that were also listed separately; DME assays to the individual SNPs also cover the haplotypes.

▪ 5 triallelic SNPs are on the list; though minor alleles for 2 are not. Two TaqMan® Assays are used to interrogate a triallelic SNP: each assay detects the major and one minor allele of the triallelic SNP [3].

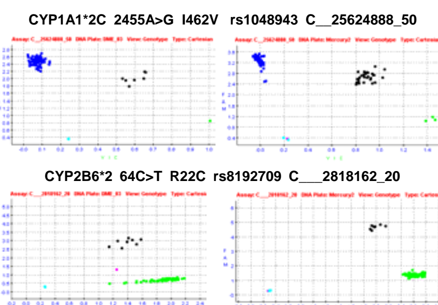
• Thus far, 164 of the 172 markers are covered by validated DME assays (95% coverage). Due to assay design challenges associated with some target sequences, >2 assay designs were tested for 66%, and ≥3 designs were tested for 31%, of the markers.

• For the 8 markers lacking DME assays (4 CYP, 3 SULT and 1 SLC gene markers), issues include challenging probe design (e.g. nearby SNP) and amplicon specificity (e.g. uncharacterized polymorphisms interfering with assay function). Further attempts will be made to design TaqMan® Assays for these, yet sequencing may be required to accurately genotype some of these markers.

Figure 1. Examples of newTaqMan® DME Assays for PharmaADME core markers

CYP1A1*2C 2455A>G I462V rs1048943 C__25624888_50

CYP2B6*2 64C>T R22C rs8192709 C___2818162_20



DME assays tested with 45 gDNAs each population: Left: African American/Caucasian; Right: Chinese/Japanese.

Table 2. A Core Marker Microsatellite Polymorphism

Gene Symbol	Allele Names	Polymorphism	dbSNP ID
UGT1A1	UGT1A1*28 _ *36 _*37	(TA)6_(TA)7_(TA)5_(TA)8	rs8175347

A fragment analysis protocol was developed to distinguish the major and 3 minor alleles of the microsatellite polymorphism in the UGT1A1 TATA-box promoter element [4] (data not shown).

TRADEMARKS/LICENSING

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Table 3: Coverage of PharmaADME CNV and Hybrid Gene Alleles by TaqMan® Copy Number Assays

Gene symbol	Allele name	Description	Assay (target)
CYP2A6	CYP2A6*4	gene deletion	Hs00010002_cn (intron 7) or exon 1 assay*
CYP2A6	CYP2A6*1X2a	duplication - breakpoint at intron 8 - 3'-UTR	Hs00010002_cn (intron 7) or exon 1 assay*
CYP2A6	CYP2A6*1X2b	duplication - Breakpoint 5.6 kb 3' of stop codon	Hs00010002_cn (intron 7) or exon 1 assay*
CYP2A6	CYP2A6*12	10 aa substitutions; Hybrid gene: CYP2A7 exon1-exon2, CYP2A6 exon3-exon9	CYP2A6 & CYP2A7 exon 1*, intron1*, Hs04488984_cn & Hs04488016_cn (intron 2), and intron 7* assays
CYP2D6	CYP2D6*5	gene deletion	Hs00010001_cn (exon 9)
GSTM1	GSTM1_Null	gene deletion	Hs02575461_cn (exon 1)
GSTM1	GSTM1*X2	gene duplication	Hs02575461_cn (exon 1)
GSTT1	GSTT1_Null	gene deletion	Hs00010004_cn (intron 1)
SULT1A1	SULT1A1_Null	gene deletion	Hs03939601_cn (intron 2)
SULT1A1	SULT1A1_XN	gene duplication	Hs03939601_cn (intron2)
UGT2B17	UGT2B17*2	deletion of 150kb region spanning UGT2B17	Hs03182490_cn (exon 1)*

* not yet commercially available

Figure 2. Location of CN Assays in CYP2A6 and CYP2A7

Figure 3. Examples of CYP2A6/CYP2A7 Copy Number Analysis

Table 4: Samples Showing CYP2A6 Copy Number Variation

GT group	Sample Name	Population	CYP2A6 CN ex1	CYP2A6 CN int1	CYP2A6 CN in2	CYP2A6 CN in7	CYP2A7 CN ex1	CYP2A7 CN in1	CYP2A7 CN in2	CYP2A7 CN in7
1	NA17111	AA	1	1	1	1	2	2	2	2
1	NA17124	Ca	1	1	1	1	2	2	2	2
1	NA17128	AA	1	1	1	1	2	2	2	2
1	NA17207	Ca	1	1	1	1	2	2	2	2
2	NA17212	Ca	1	1	2	2	2	2	1	1
2	NA17214	Ca	1	1	2	2	2	2	1	1
2	NA17242	Ca	1	1	2	2	2	2	1	1
3	NA17115	AA	2	2	3	2	2	2	1	2
4	NA17217	Ca	2	2	2	1	2	2	2	3

TaqMan® Copy Number Assays designed to specifically target CYP2A6 or pseudogene CYP2A7 exon 1, intron 1, intron 2 and intron 7 sequences were tested on 45 each African American (AA) and Caucasian (Ca) gDNAs. Results: 81 samples had 2 copies of both CYP2A6 and CYP2A7; 9 samples had copy number variation within CYP2A6 that fell into 4 genotype (GT) groups, consistent with:

[1] 1 copy CYP2A6*4 deletion; 1 copy CYP2A6; 2 copies CYP2A7

[2] 1 copy CYP2A6*12 (CYP2A7exons 1-2 /CYP2A6 exons 3-9); 1 copy each CYP2A6 & CYP2A7.

[3] 2 copies each CYP2A6 & CYP2A7; 1 copy each with gene conversion of exon 3

[4] 2 copies each CYP2A6 & CYP2A7; 1 copy each with gene conversion of intron 7

Note: GT group 3 and 4 variants are not reported on the Cytochrome P450 (CYP) Allele Nomenclature Committee website [5].

Note: 10 samples (6 AA and 4 Ca) showed only 1 copy of the CYP2A7 in1 target and 2 copies of the other 7 targets, suggesting the presence of a relatively frequent deletion or other polymorphism in CYP2A7 intron 1.

Table 5. Observed Minor Allele Frequencies of CYP2A6 alleles

CYP2A6 allele	AA	Ca	total
CYP2A6*1x2	0	0	0
CYP2A6*4	0.033	0.011	0.022
CP2A6*12	0	0.033	0.017

CONCLUSIONS

- Summary of PharmaADME core marker list coverage by TaqMan® DME and CN Assays:
  -- 164 of 172 SNP/InDels covered by DME assays
  -- 10 of 10 CNV markers covered by 6 CN assays
  -- 1 gene hydrid covered by 4-8 CN assays
  -- 1 microsatellite marker - requires a fragment analysis assay
  -- 175 of 184 total with TaqMan® Assays = 95% coverage
- For the 5 triallelic SNPs on the core list, a protocol was developed whereby paired TaqMan® DME Assays are used to genotype the 3 SNP alleles [3].
- TaqMan® CN Assays detect core gene duplications, deletions, and hybrids. Multiple CN assays may be required to discern all gene alleles (e.g. CYP2A6).
- TaqMan® DME and Copy Number Assays to core PharmaADME markers provide a simple, scalable method for accurate genotyping of these important alleles in pharmacogenetic research.

REFERENCES

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4. UGT1A1*28_*36_*37 fragment analysis protocol document, in progress.
5. Cytochrome P450 Allele Nomenclature web site: www.cypalleles.ki.se