Detecting Polymorphisms in Drug Metabolism Genes Uncovers More Than Genotypes

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ABSTRACT

Polymorphisms within drug metabolism pathways have been difficult to study due to the complexity of genetic information associated with these genes. Many of the genes within these pathways are part of large gene families that include several pseudogenes, thus generating large homology barriers that are difficult for most technologies to overcome. Additionally, many of the polymorphisms that are identified in public SNP databases do not use common genomic identifiers that allow researchers to readily understand which polymorphisms they are dealing with.

To overcome these barriers, Applied Biosystems has developed an extensive collection of TaqMan® Genotyping Assays to detect single nucleotide polymorphisms (SNPs), multiple nucleotide polymorphisms (MNPs), and insertion/deletions (in/dels) within 220 drug metabolism genes. The polymorphisms identified for this collection are within regulatory elements, coding regions, or splice junctions. Each assay was wet-lab tested on 180 DNA samples from 4 human populations, (Caucasian, African American, Chinese, and Japanese) to estimate the minor allele frequency in each of these populations and all assays were checked for Hardy-Weinberg Equilibrium. During the performance testing of the assays, many of the genes in this collection were identified as having unique characteristics, for example, gene deletions or amplifications (GSTM1 and CYP2D6) that can affect the interpretation of results from SNP genotyping assays. It is important to understand the underlying biology of particular genes when interpreting results from genotyping experiments and when evaluating how well a particular genotyping technology performs.

INTRODUCTION

Targeting over 2000 polymorphisms spanning 220 genes, the Applied Biosystems TaqMan® Drug Metabolism Genotyping Assays are the industry's most comprehensive collection to date. The extensive bioinformatics used to design these assays and the extensive wet-lab validation provides a high level of genotyping confidence.

Genotyping results, at times, can be confounded by other factors specific to certain drug metabolism genes. Thus, it is very important to understand those factors when it comes time to interpret the data. Throughout the validation process, we encountered many assays where the results were difficult to interpret due to the presence of: gene deletions, tri-alleles, gene duplications, and gender differences.

Whenever possible, data is provided as support tools for customers using an assay from the TaqMan® Drug Metabolism Genotyping Assay Collection.

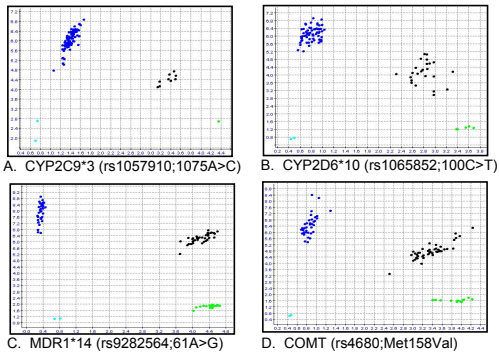
MATERIALS AND METHODS

All polymorphisms were mapped to the human genome assembly. The mapping identified other polymorphisms in close proximity to the target SNP. The nearby polymorphisms were then masked. The masking ensured that primers and probes were not designed over other SNPs. All designs underwent *in-silico* QC, to ensure primers did not have high identity to homologous regions in the genome

Assays were then wet-lab tested to ensure optimal performance. Assays were run on genomic DNA (gDNA) from four human populations: Caucasian, African-American, Chinese, and Japanese (forty five unique individuals from each population). PCR was performed on a GeneAmp® PCR System 9700 thermalcycler and an endpoint read on an ABI PRISM® 7900HT SDS instrument.

RESULTS

Figure 1. Typical Drug Metabolism Genotyping Assay Results

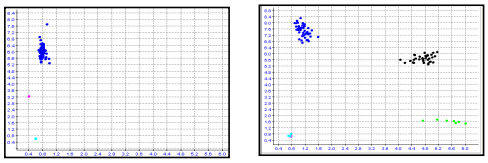


A. CYP2C9*3 (rs1057910;1075A>C) B. CYP2D6*10 (rs1065852;100C>T)

C. MDR1*14 (rs9282564;61A>G) D. COMT (rs4680;Met158Val)

Figures 1a-d represents typical genotyping results for 4 important polymorphisms in drug metabolism genes. The plots are representative of the high genotyping confidence the assays provide. Even with the challenging targets in CYP2D6 (B), the assay yielded robust results.

Figure 2. Low Minor Allele Frequency (MAF) Polymorphisms: ALDH2 (rs671)



A. Caucasian/African-American B. Chinese/Japanese

allele frequencies		
	allele 1	allele 2
AA	0%	100%
CAU	0%	100%
Chinese	32%	68%
Japanese	23%	77%

Table 1. Allele frequencies for TaqMan® Drug Metabolism Genotyping Assay for rs671.

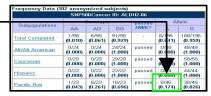
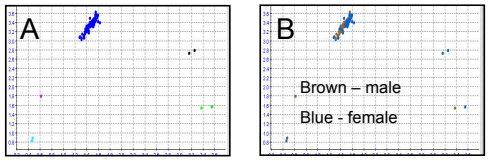


Table 2. Allele frequencies for rs671 from SNP500Cancer website.

Not all populations we tested show the minor allele. With some polymorphisms, the minor allele frequency may be so low, less than 5%, that it was not expected to appear in our tested population.

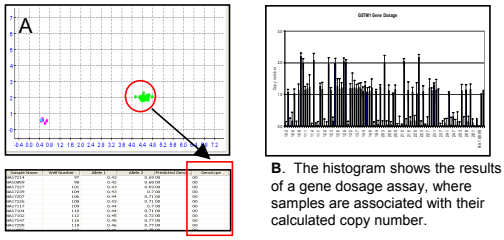
ALDH2; rs671, the minor allele frequency does not show in the Caucasian/ African-American samples (A), but the minor allele does appear in the Chinese / Japanese population (B). Our MAF, when compared to that of the SNP500Cancer Database (http://snp500cancer.nci.nih.gov) is very similar. The discrepancy lies in the differing Asian populations makeup of their set.

Figure 3. Behavior of SNPs on the X chromosome: MAOB (rs6324)



Typically, all assays were evaluated for Hardy-Weinberg Equilibrium. The above example (A) represents an assay that did not meet HWE, as there are an equal number of heterozygotes as minor allele homozygotes. The expected number of minor allele homozygotes should be less than the number of heterozygotes. Further investigation revealed (B) that the assay was to a gene on the X chromosome. In these cases all heterozygotes should be females.

Figure 4. Gene deletions: GSTM1



B. The histogram shows the results of a gene dosage assay, where samples are associated with their calculated copy number.

Non-amplification of certain samples run using a GSTM1 SNP assay (A) lead to a gene dosage assay to verify copy number[3]. Samples that showed zero copies (B) corresponded to the non-amplified samples in the TaqMan® assay. Understanding that the target was within the GSTM1 gene explains why individuals in certain populations are not amplifying as they have the deletion in both alleles[1].

Figure 5. Gene duplication: CYP2D6*17

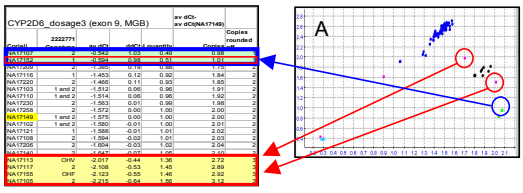


Table 3. Results of real-time gene dosage assay for CYP2D6*17 polymorphism.

Outliers (A) represent possible copy number polymorphisms that are possible in CYP2D6 gene. Presence of outliers and an understanding of the CYP2D6 gene, necessitated further investigate of the results. Copy number differences were confirmed by real-time gene dosage[3] results listed in Table 3. Variation in copy number for the CYP2D6 gene correspond to altered phenotypes, such as poor, intermediate, extensive, or ultra-rapid metabolizers[2].

CONCLUSIONS

- Extensive bioinformatics and wet-lab validation provides the TaqMan® Drug Metabolism Assay Collection a high level of genotyping confidence, even for difficult to design targets that have homology to other regions in the genome.
- Even with robust assays, understanding the biology that underlies these targets is key to the correct interpretation of the genotyping results. TaqMan® Drug Metabolism assays can produce quantitative results for copy number polymorphisms. No other genotyping technology can produce such results, providing clues to other underlying gene polymorphisms.
- Outliers may be evidence of an underlying inter-individual difference, such as gene duplication. Extra copies of genes, such as CYP2D6, may correspond to an extensive drug metabolizer phenotype.
- Deletions resulted in non-amplification of samples. Samples that drop out correspond to individuals who have the null allele.

REFERENCES

1. Xu, et al. *Journal of Biological Chemistry* 273, 6 (1998)
2. Endrizzi, et al. *Analytical Biochemistry* 300 (2002)
3. Schaeffeler, et al. *Human Mutation* 22 (2003)

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TRADEMARKS/LICENSING