Identification of Novel CYP2D6 Haplotypes that Interfere with TaqMan Copy Number Analysis

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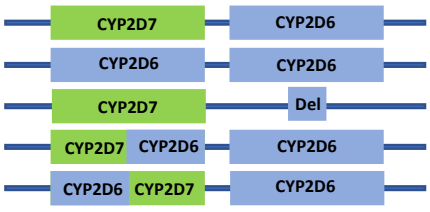
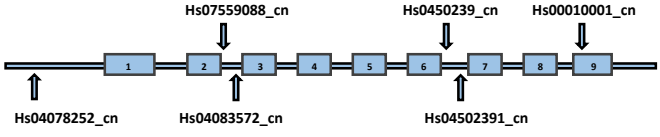
Background

Cytochrome P450 2D6 (*CYP2D6*) is a highly polymorphic gene encoding an enzyme critical in the metabolism of up to 25% of commonly prescribed drugs[1]. There are over 100 described star (*) alleles, including gene conversions, dup/dels and gene-fusions (D6/D7 and D7/D6 hybrids), many of which can cause altered enzyme activity **(Figure 1)**. Accurate copy number (CN) calling is critical in determining patient drug response[2-4].

Figure 1. *CYP2D6* and *CYP2D7* Structural Arrangements. *CYP2D6* is located next to the highly homologous *CYP2D7* pseudogene. This has led to the generation of stable duplications, deletions and hybrid alleles.



TaqMan CNV assays (Thermo Fisher Scientific) utilize specific primers and labeled probes to evaluate the CN state of a genomic region of interest. Polymorphisms within the primer or probe target sequence can generate a false positive for a CN loss. Three *CYP2D6* intra-gene regions can be tested using commercially available TaqMan CNV assays: intron 2, intron 6 and exon 9 (**Figure 1**). Hybrid genes or conversions are detected as discrepant CN calls between the three probe locations.



CYP2D6 CN Assays	Location	GRCh38 Location
Hs04078252_cn	5'UTR	Chr.22:42131642
Hs04083572_cn	Intron 2	Chr22:42129681
Hs07559088_cn	Intron 2	Chr22:42129479
Hs04502391_cn	Intron 6	Chr22:42127680
Hs04502394_cn	Intron 6	Chr22:42127759

Figure 2. Representation of *CYP2D6* gene layout and probe position.

Methods and Results

The TaqMan CNV assays shown in **Figure 1. A** set of 20 samples with CN inconsistencies between the CNV probes were idnetified. We performed long-range PCR (XL-PCR), Next-Gen sequencing (NGS), Droplet Digital™ PCR (ddPCR™; BIO-RAD) and allele-specific Sanger sequencing. For NGS, the entire *CYP2D6* region was amplified for each sample using primers described by Black et al[5]. NGS libraries were prepared from the XL-PCR products and sequenced on the Ion Proton System (Thermo Fisher Scientific). Sequence analysis[6,7] identified three SNPs in intron 6 and four in intron 2 that are located within the TaqMan probe/primer binding sites. These SNPs were only present in samples that showed a Taqman CN loss at their respective genomic location.

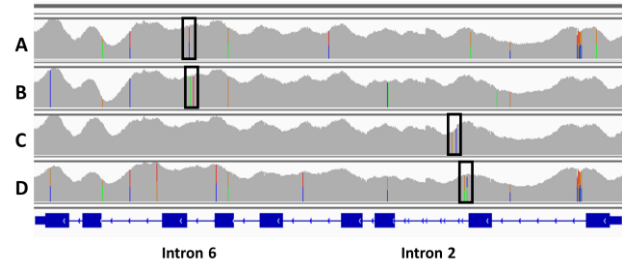


Figure 3. Sequence alignment to *CYP2D6*. Representative alignments of the seven SNPs in probe or primer binding sites. Samples **A** and **B** were found to have an intron 6 CN loss with probe Hs04502391. Samples **C** and **D** showed a CN loss in intron 2 with probe Hs04083572. The SNPs identified within the probe region are indicated in black boxes.

Methods and Results

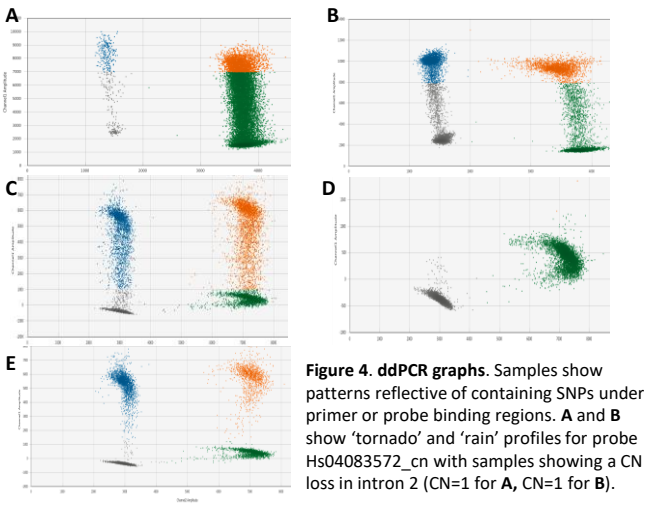


Figure 4. ddPCR graphs. Samples show patterns reflective of containing SNPs under primer or probe binding regions. **A** and **B** show 'tornado' and 'rain' profiles for probe Hs04083572_cn with samples showing a CN loss in intron 2 (CN=1 for **A,** CN=1 for **B**). **C** 'Tornado' and **D** zero amplification for Hs04502391_cn with samples showing a CN loss in intron 6 (CN=1 for **C** and CN=0 for **D**). **E** Representative CN=2 control result for probe Hs04502391_cn.

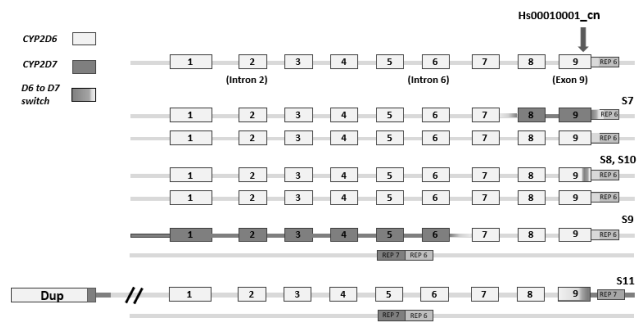


Figure 5. *CYP2D6-CYP2D7* Hybrid alleles with exon 9 CNV. Representative figures of four alleles with *CYP2D6-CYP2D7* hybrids. Sample S7 contained a conversion to *CYP2D7* that included exons 8 and 9, with a REP6 region. Samples S8 and S10 were found to contain a exon 9 *CYP2D7* conversion and REP6 region. Sample S11 contains a conversion to *CYP2D7* in exon 9 and has a REP7 region.

Conclusions

In total, five novel haplotypes with SNPs within the intron 2 and 6 probe/primer binding sites were identified: **1* sub-allele 1: rs770138443, rs78854695; **1* sub-allele 2: rs781257354, rs375222401; **2* sub-allele 1: rs180847475, rs186133763; **2* sub-allele 2: rs370010370; **2* sub-allele 3: rs539070953. In addition, four distinct alleles containing a *CYP2D7* conversion that included exon 9 were identified.

In summary, performance of widely used TaqMan CNV assays is affected by the presence of sequence variations within the probe or primer binding sites. The identification of these haplotypes and further characterization of additional variations will be important to accurately determine *CYP2D6* genotype to guide drug therapy.

References

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6) James T. Robinson, Helga Thorvaldsdóttir, Wendy Winckler, Mitchell Guttman, Eric S. Lander, Gad Getz, Jill P. Mesirov. **Integrative Genomics Viewer**. Nature Biotechnology 29, 24–26 (2011) Dunnenberger et al. Annu Rev Pharmacol Toxicol. 2015;55:89-106
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