Characterization of CYP2D6 structural variants containing the *CYP2D6*68* hybrid

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Background

CYP2D6 is a highly polymorphic gene encoding protein responsible for the metabolism or bioactivation of over 20% of clinically used drugs[1,2]. In addition to gene duplications and deletions, the gene locus is also afflicted by structural rearrangements that arose through recombination with the *CYP2D7* pseudogene (collectively referred to as copy number variations, or CNVs). *CYP2D6-CYP2D7* and *CYP2D7-CYP2D6* 'hybrid' genes can occur in different configurations. The most commonly observed is the *CYP2D6*68+*4* tandem in which *CYP2D6* switches to *CYP2D7* in intron 1 rendering the **68* gene product nonfunctional[1-4].

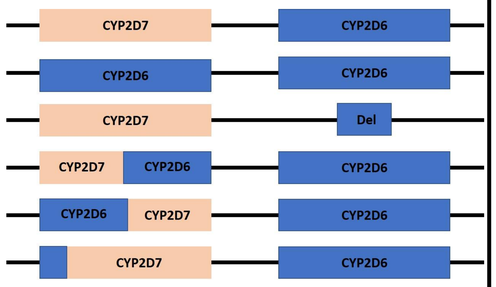


Figure 1. CY2D6 and CYP2D7 structural arrangements. *CYP2D6* is located next the highly homologous pseudogene CYP2D7. This has led to the generation of stable duplications, deletions and D6/D7 hybrid alleles.

Methods

RPRDx:

- Samples from St. Jude's Children's Research hospital (n=500) were genotyped on the Pharmacoscan™ array.
  - In addition to SNPs, the array also detects CNVs in the 5', 3' and exon 9 regions facilitating the detection of hybrid gene copies.
  - Long range (XL) PCR and Next Generation Sequencing (NGS) was performed on selected samples to characterize these rearrangements in more detail.

Children's Mercy:

- Samples from Children's Mercy were genotyped using TaqMan assays and CNVs determined with a quantitative CNV assay and XL-PCR
  - Samples with inconsistent genotyping results and a tentatively having a *CYP2D6*68+*4* were further characterized by allele-specific XL-PCR, Sanger sequencing and digital droplet PCR (dd) PCR.

PharmGenetix:

- Samples from PharmGenetix were characterized by allele-specific XL-PCR and Sanger sequencing.
  - CNV was determined in the 5', intron 2 and exon 9 regions with ddPCR.

*CYP2D6*68* Alleles

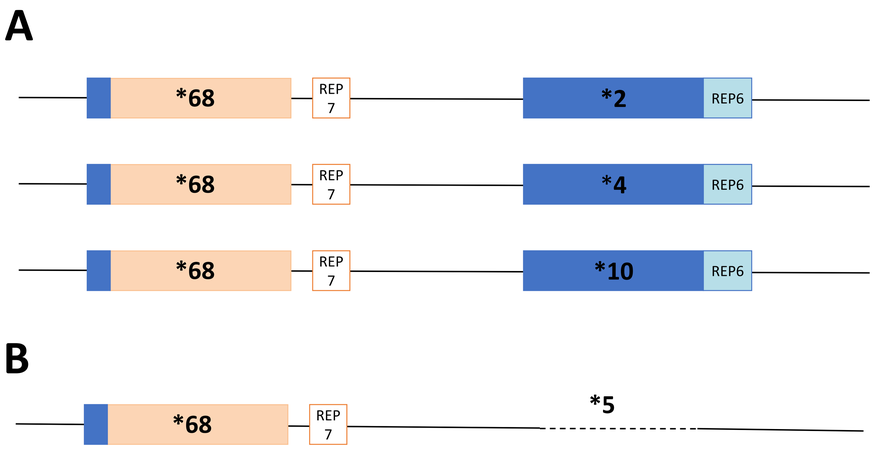
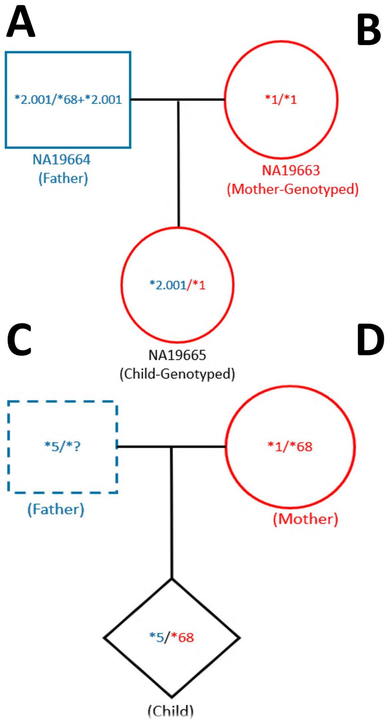


Figure 2. structural variants detected. (A) The *CYP2D6*68* allele was detected in tandem arrangement with **2, *4* and **10* gene copies. (B) The *CYP2D6*68* allele was also detected as a 'singleton', i.e. it was not accompanied by another gene copy. This allele carries a *CYP2D7*-like downstream region (REP7) which supports amplicon formation with a commonly used XL-PCR assay detecting the *CYP2D6*5* gene deletion. Thus, a singleton *CYP2D6*68* may cause false-positive *CYP2D6*5* results



B

ddPCR CYP2D6 CNV Assay	Copy Number
5' UTR	3
Intron 6	2
Exon 9	2

D

Diagnostic Assays	Mother's Results	Child's Results
*5 XL-PCR Assay (CYP2D6 Deletion)	Positive	Positive
Fragment A (CYP2D6 Gene Present)	Positive	Negative
ddPCR CNV *CYP2D6* 5' UTR	2	1
ddPCR CNV *CYP2D6* Intron 6	1	0
ddPCR CNV *CYP2D6* Exon 9	1	0

Figure 3. Characterization of *CYP2D6*68* arrangements. In addition to SNP genotyping, inheritance (family pedigree), quantitative copy number assays targeting different gene regions, and sanger sequencing of XL-PCR products (not shown) were utilized.

Haplotype Frequencies

RPRDx:

- The sample cohort (n=500) represents an ethnically mixed clinical population
  - The vast majority of *CYP2D6*68* were found in tandem with a *CYP2D6*4* gene copy but was also detected in tandem with a *CYP2D6*2* or **10*.

Children's Mercy:

- Consistent with the RPRDx findings, the *CYP2D6*68+*4* tandem was the most common observed
  - Two investigated samples had a *CYP2D6*68+*2* tandem and one was a *68 singleton.

PharmGenetix:

- Again, the *CYP2D6*68+*4* tandem was the most common observed, occurring in 32% of the samples positive for **4*.
  - Two novel **68*-like hybrids were also discovered, one in tandem with **4* and one with **1*.

Table 1. Frequency of *CYP2D6*68*.

Testing location and study size	*68+*2	*68+*4	*68+*10	*68 Singleton
RPRDx (n=500)	3 (0.6%)	54 (10.8%)	4 (0.8%)	0 (0.0%)
Children's Mercy* (n=N/A)	2	n/a (~5%)	0 (0.0%)	1
Pharmgenetix (n=900)	1 (0.11%)	103 (11.4%)	0 (0.0%)	0 (0.0%)

*Samples were from multiple projects involving subjects with different ethnic backgrounds

Conclusions

- To date, the *CYP2D6*68* hybrid has only been described in tandem with **4*, which led to the practice of *CYP2D6*68+*4* default assignments.
- This is the first report describing *CYP2D6*68+*2* and **68+*10* tandems and a **68* singleton.
- The occurrence of *CYP2D6*68* in non-**4* configurations can potentially result in incorrect diplotype assignments.
- The *CYP2D6*68* hybrid harbors g.100C>T which is present on numerous other haplotypes including *CYP2D6*10* and thus may interfere with genotype test interpretation and translation of genotype to phenotype[2,3,6].

References

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