Genetics inMedicine | CORRESPONDENCE

Challenges in clinical implementation of *CYP2D6* genotyping: choice of variants to test affects phenotype determination

Cavallari et al. on behalf of the Implementing GeNomics In pracTicE (IGNITE) Network recently reported the experiences of eight institutions with *CYP2D6* genotyping implementation.[1] Although there were some similarities, there were also important differences in the *CYP2D6* alleles that could be detected across institutions. To illustrate the potential pitfalls of this lack of standardization in *CYP2D6* testing, we report a recent patient case of incongruous *CYP2D6* genotype results when genotyped by different assays.

An 18-year-old male patient of black race with sickle cell disease (SCD-HbSC) was enrolled on St. Jude Children's Research Hospital's PG4KDS clinical implementation of pharmacogenetics trial.[2] Genotyping was performed on the DMET™ Plus platform (ThermoFisher, Waltham, MA, USA) by the reference laboratory (Medical College of Wisconsin, Milwaukee, WI, USA). The genotyping probe for *rs3892097* used to call the *CYP2D6 *4* allele failed, resulting in an inconclusive *CYP2D6* diplotype of either *(*4/*40)2N* or *(*10/*40)2N* that would translate to a poor metabolizer (PM) or intermediate metabolizer (IM) phenotype, respectively, using the *CYP2D6* activity score method.[3] To resolve the **4* probe failure, a single-gene *CYP2D6* test (ARUP Laboratories, Salt Lake City, UT, USA) was ordered. The result was reported as a **4/*17* diplotype translating to an IM phenotype. This single-gene *CYP2D6* assay was not designed to detect the **40* allele, however. While considering these seemingly discordant results, we hypothesized that the patient likely had one copy of the **4* no function allele and one copy of the **40* no function allele, predicting a PM phenotype. The patient was prescribed analgesics that were not subject to significant *CYP2D6* gene–drug interactions while a definitive genotype was confirmed by another method. The blood sample was then genotyped on the PharmacoScan™ assay (ThermoFisher; RPRD Diagnostics LLC, Milwaukee, WI, USA) confirming the *CYP2D6 (*4/*40)2N* diplotype, and the patient was assigned a final phenotype of CYP2D6 PM. Upon confirmation of the genotype, the patient's health record was updated. The patient and his health-care team were informed of the high-risk CYP2D6 phenotype and its clinical implications (i.e., potential lack of analgesic effect). This patient is at risk for recurrent episodes of vaso-occlusive pain crises throughout his life due to his SCD diagnosis. He is likely to require opioid analgesia in the future, thus making an accurate CYP2D6 phenotype assignment imperative for his clinical care.

The *CYP2D6 *40* allele differs from the **17* allele by the presence of an in-frame insertion variant *rs72549356*.[4] If this variant is not specifically probed, the "default" call becomes **17*. This possibility is noted in the Clinical Pharmacogenetics Implementation Consortium (CPIC) *CYP2D6*-related guidelines' supplemental materials[3,5] and by others.[6] We report here a true patient case where this difference had clinical relevance. The **17* allele is a decreased function allele assigned an activity score of 0.5 while the **40* allele is a no function allele assigned an activity score of 0.[3] In this patient's case, the differing reported genotypes would have translated to different phenotype assignments (i.e., IM for a *CYP2D6 *4/*17* genotype versus PM for a *CYP2D6 *4/*40* genotype) and consequently different prescribing recommendations. Indeed, prescribing recommendations from 5 of 6 CPIC guidelines are different for CYP2D6 IMs and PMs for 12 drugs.[5] As of 6 May 2019, there are an additional 20 drugs with *CYP2D6* interactions that are considered to have sufficient evidence for at least one prescribing action to be recommended (CPIC levels A or B).[7]

The *CYP2D6 *40* allele is most commonly observed in the African population with an average allele frequency of 0.017.[3,5,6] The **17* allele is more frequent in this population, with an average allele frequency of 0.2, but this may be overestimated due to undertesting of the **40* and **58* defining variants leading to a "default" **17* allele assignment.[3,6] The **17* and **40* allele frequencies in many African ancestry groups suggest that if *rs72549356*, which discriminates the **40* allele from the **17* allele, is not interrogated, approximately 1 of 12 patients with a called **17* decreased function allele could actually carry the **40* no function allele. Such patients could potentially be assigned a consequent inaccurate phenotype, a frequency we consider to be intolerably high.

Based on reported methods used, most institutions that have implemented *CYP2D6* genotyping[1] would have assigned an inaccurate phenotype to this patient. Our case highlights the importance of standardizing variants interrogated across laboratories, and establishing a "must test" set of alleles[8] for clinically important pharmacogenes that should consider allele frequencies by race/ancestry groups, particularly when the variants discriminate between alleles with different functions. Such a "must test" list was recently described for *CYP2C19* and *CYP2C9*, related and clinically important pharmacogenes.[9,10]

We report our case as an example of the challenges and potential consequences of variability in *CYP2D6* genotyping assay design and selection. Important considerations for adequate allelic coverage remain, particularly when they

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impact phenotype assignment and consequent prescribing recommendations.

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DISCLOSURE

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