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Impact of CYP2D6 Ultra Rapid Metabolism on Therapeutic Drug Efficacy in Type 2 Diabetic Patients

A Pineda[1], N Williams[1], N Chaudhry[1,2], M Pietruszka[1,3], K Daldalyan[1], Y Valles-Ayoub[1], M Robinson[1,4*]

*[*1]Firmalab,USA.*

[2]California State University Northridge, USA.

[3]USC Keck School of Medicine, USA.

[4]Marina Diabetes Center, Marina del Rey, California, USA.

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ABSTRACT

Duplication of the CYP2D6 allele results in excess enzyme activity and can alter the effects of administered drugs. This ultra-rapid metabolizer phenotype and its impact on conventional treatment of Type 2 Diabetes Mellitus is the focus of the present communication. Genomic DNA was extracted from buccal swab samples taken from 215 patients and analyzed for CYP2D6 activity. Results indicated that 22 patients (10.2%) were positive for CYP2D6 duplication. Of these 22 ultra-rapid metabolizers, 12 patients (54.5%) were poor responders to conventional Type 2 Diabetes treatment. These results may reflect the small population size used in the study. A larger sample with a focus on the mechanism of drug metabolism in the future may elucidate a more causal relationship between CYP2D6 duplication and optimized therapeutic outcome of conventional Type 2 Diabetes treatment regimens.

Keywords: Cytochrome P450, CYP2D6, Ultra rapid metabolizers, Diabetes, Pharmacogenetics

Abbreviations: CYP450: Cytochrome P450; CYP2D6: Debrisoquine 4-hydroxylase; EM: Extensive Normal; UM: Ultra-rapid metabolizer; PM: Poor metabolizer; T2D: Type 2 Diabetes

INTRODUCTION

Cytochrome P450 (CYP450) enzymes are essential for the metabolism of drugs. They account for around 75 percent of enzymes involved in drug metabolism [1]. An important cytochrome P450 (CYP) enzyme, CYP2D6 (debrisoquine 4-hydroxylase), represents 1-5% of the CYP liver content and is responsible for the hydroxylation or de-alkylation of up to 25% of commonly prescribed drug classes drugs such as analgesics, anticonvulsants, antidepressants, antipsychotics, opioids, antiarrhythmics and tamoxifen, many of which have a narrow therapeutic window [2-5].

CYP2D6 is highly polymorphic and allele variations alter the enzyme's rate of drug metabolism [6]. These differing rates of metabolism are categorized into the following phenotypes: extensive normal (EM), ultra-rapid metabolizers (UM), intermediate metabolizers (IM) and poor metabolizers (PM) [7]. Poor metabolizers have a complete or near-complete loss of CYP2D6 function while ultra-rapid metabolizers have multiple copies of the gene and therefore metabolize their substrates ultra-rapidly [8]. These changes in metabolism may cause alterations to the therapeutic effect of the drugs being metabolized and the impact can be clinically significant [9-11]. For example, a poor metabolizer is unable to convert codeine into its biologically active form, morphine, thereby eliminating its therapeutic effect [12]. On the other side of the spectrum, giving a normal dose of an opioid to an ultra-rapid metabolizer could result in excessive drug effect and increased activity of the CYP2D6 variants thus resulting in toxic levels of potent metabolites of the parent drug [13] and accumulation of drug in breast milk [14,15].

A compilation of data from across the world concerning CYP2D6 predicted PM prevalence to be between 0.4-5.4%, IM between 0.4 and 11%, EM between 67-90% and UM

Corresponding author: Michael F Robinson, MD, Firmalab, CPI Director, Marina Diabetes Center, Marina del Rey, California, Tel: 310-863-8; E-mail: mfrobinsonmd@gmail.com

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between 1 to 21% [16]. In addition, there were significant variations of allele prevalence between ethnic groups [17,18]. Prevalence variability across race groups specifically of UMs has been well studied. Some notable UM prevalence's include Ethiopians (29%) [19], Saudi Arabians (21%) [20-22], Spaniards (10%) [23], Turkish (5.6%) [24], African Americans (4.9%) [25-28] Caucasion Americans (4.3%) [25], Croatians (4%) [29], Colombians (1.9%) [30], European white populations (0.8-1.0%) [31-33], and Chinese (0.9%) [34,35].

CYP2D6 duplication in relation to Type 2 Diabetes Mellitus (T2D) is not well understood. T2D is a major public health concern with prevalence reaching 8.5% in adults in the United States [36]. The International Diabetes Federation estimates that, worldwide, 425 million adults were living with diabetes mellitus in 2017, with about 90% of those cases being Type 2 [37]. Projections report that this number may reach 629 million by 2045 [37]. It has been demonstrated that T2D has the ability to alter CYP450 enzyme activity, which in turn impacts the patient's response to treatment [38,39]. Understanding the role of CYP2D6 duplication in patients with T2D may be useful for personalized treatment and improvement of therapeutic outcomes [40,41].

MATERIALS AND METHODS

Nucleic acid isolation

Buccal epithelial cells were obtained with the Hydra Flock 6" Sterile Elongated Flock Swab w/Plastic Handle & Dry Transport Tube (Puritan Medical Products, Glendora, United States, Cat. #25-3606-H BT.) Cellular DNA was isolated using the Quick-DNA Miniprep Plus Kit (Zymo Research, Irvine, United States, Cat. # D4068) following manufacturer's instructions. DNA optical density was measured using the ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, United States) for concentration determination. After DNA quantitation, DNA was diluted using RNAse free H_2O to a concentration of 20 ng/µl. Isolated DNA was stored at -20°C as necessary.

PCR

To identify individuals with a duplicated CYP2D6 gene, nucleic acid is amplified using primers designed to amplify a 3200 bp segment (CYP-17f: 5'-TCCCCCACTGACCCAACTCT-3') CYP-32r: 5'-CACGTGCAGGGCACCTAGAT-3'). Patient samples are amplified alongside a positive control obtained from Coriell in a Techne TC-412 Thermal Cycler. Each 12.5 µl reaction was comprised of 7.02 µl RNAse free H_2O, 1.25 µl 10X Long Range PCR buffer, 0.63 µl 10 mM dNTP mix, 1 µl 10 µmol of primer mix (CYP207F/32R), 0.1 µl Qiagen Long Range enzyme, and 2.5 µl (50ng total) DNA. The thermocycler program consists of a 3 minute enzyme activation step at 93°C, followed by 35 PCR cycles, each of which included three steps: denaturation of DNA template and primers for 15 seconds at 93°C, annealing of primers to single-stranded DNA template for 30 s at 62°C, and extension of amplicon strand (complementary to DNA template strand) for 5 min at 68°C. The PCR included a final holding temperature at 4°C.

Gel electrophoresis and imaging

Each gel was electrophoresed with 3 µl Kapa Express DNA Ladder kit (Kapa Biosystems, Boston, United States) as a molecular weight marker. Kapa loading dye (6X) was added to each PCR tube containing a total reaction volume of 12.5 µl. 10 µl of each amplicon was loaded into a 1% Lonza Reliant Minigel TAE (Lonza Group AG, Basel, Switzerland, Cat. #54801) and electrophoresed at 90V for 30 minutes. The gels were stained in a 25 µl Sybr®-Safe DNA gel stain (Thermo Fisher, Carlsbad, United States, Cat. #S33102) and 250 µl 1X TBE buffer bath. The gels were then viewed and imaged in a UVP Epi Chemi II Darkroom (UVP, United States) imaging system and analyzed for amplification of the duplicated CYP2D6 region. A positive signal for CYP2D6 duplication yields a 3200 bp band **(Figure 1)**.

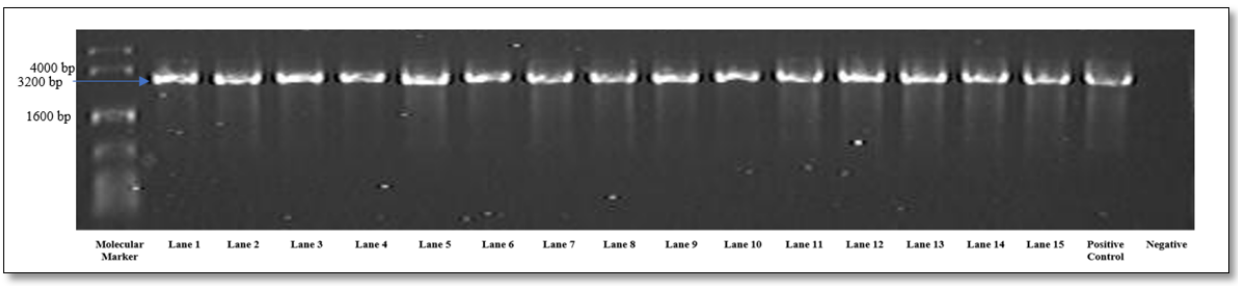

Figure 1. Image of gel electrophoresis. The arrow indicates a 3200 bp band.

Sequencing analysis

The duplicated patients' CYP2D6 exons 1, 2, 3, 4, 5, 6, 7, and 9 genotypes were analyzed for other variations via target exon sequencing. The target exons were Sanger sequenced using ABI 3130xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and cross-referenced to NCBI Reference Sequence NC_000022.10 [42], using Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA) for analysis [43].

RESULTS

Images obtained of the gel electrophoresis using UVP Epi Chemi Darkroom and Lab works Image Acquisition and Analysis software, identified 22 patients out of 215, positive for CYP2D6 duplication. A positive signal was denoted by a 3200 bp band found on the gel as denoted in **Figure 1**. This yielded a 10.2% rate of ultra-rapid metabolizers in a sample of 215 patients. Using further analysis, it was discovered that 54.5% of the 22 patients were also poor responders to conventional type 2 diabetes treatment regimens. Sequencing analysis revealed that 45% of the CYP2D6 duplicated patients also had at least one additional variation in conjunction with CYP2D6 duplication. These variations included CYP2D6*2, CYP2D6*3, CYP2D6*4, CYP2D6*10, CYP2D6*17, and CYP2D6*41. Five of the twenty-two (22.7%) CYP2D6 duplicated patients specifically had the CYP2D6*10 polymorphism (**Figure 2**).

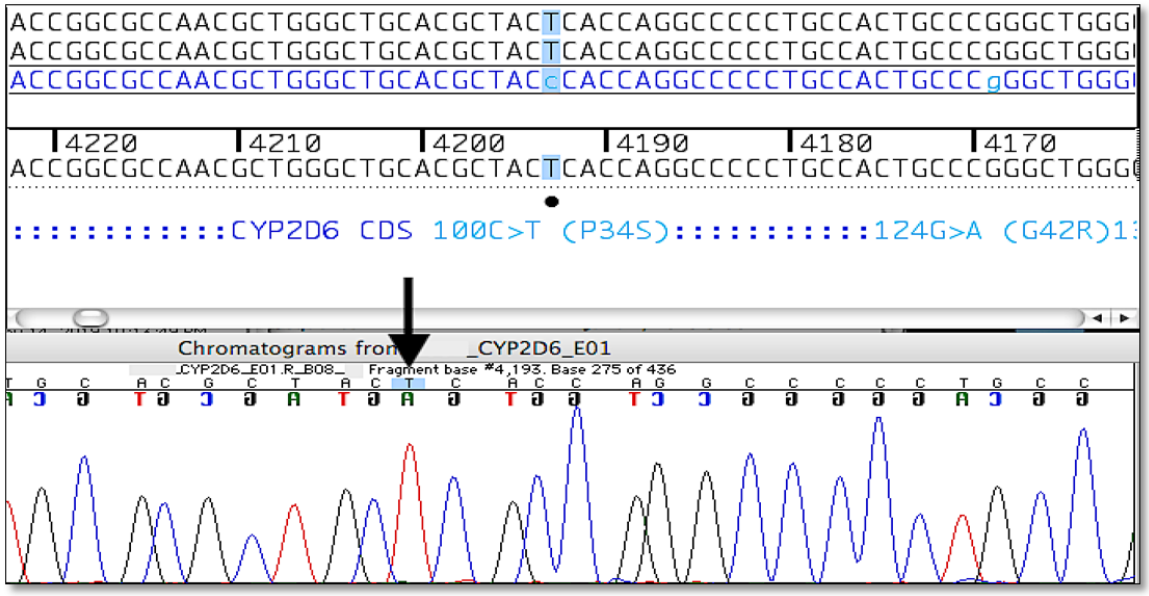

Figure 2. Electropherogram for a CYP2D6 duplicated patient who also has a homozygous CYP2D6*10 genotype (Gene Position: 100C>T, Bp Position c.100C>T, AA change: p.P34S) [43], [44]. The arrow marks the variation.

DISCUSSION

Our findings suggest that CYP2D6 genotyping of diabetic patients may have clinical significance in prescription planning with respect to therapeutic efficacy. To the extent that phenotypic expression of CYP activity corresponds to CYP genotype, it may be possible to design optimized therapeutic regimens for selective CYP substrates based on knowledge of a patient's CYP genotype.

CYP2D6 is fixed in the phospholipid bilayer of the endoplasmic reticulum. Switching between the opened and closed conformations allows for substrate access to the active site [45,46]. The active site of CYP2D6 is bordered by iron-protoporphyrin IX and lined by amino acid residues, which can bind numerous substrates with distinct features [46,47]. This accounts for the ability of CYP2D6 to metabolize a broad range of substrates. The substrates are typically lipophilic bases with a planar hydrophobic aromatic ring and a nitrogen atom [46]. Polymorphisms affecting polypeptide sequences that compose active sites change the enzyme's ability to bind substrates and thereby affect observed metabolic phenotype. It is possible that a polymorphism that changes the binding pocket of the enzyme [45] combined with duplication of CYP2D6 can alter one's ability to metabolize medications used in conventional diabetes treatments. Future studies should seek to understand which medications could be most impacted within diabetes treatment regimens by a duplication of CYP2D6. Additionally, a more in depth look at patient demographics such as age, sex, ethnicity and disease duration should be undertaken and presented in future studies.

CONCLUSION

Testing for CYP2D6 for a more customized prescription planning is being used increasingly in clinical practice. This research has revealed a possible link between CYP2D6 duplication and treatment response in patients with Type 2 Diabetes Mellitus. Our findings suggest that CYP2D6 variations may alter metabolic responses to diabetes treatment. CYP2D6 duplication in conjunction with other CYP2D6 variations produce various phenotypes with ranging degrees of functionality. Following genetic analysis, it is important to determine the phenotype by referencing CPIC guidelines in order to adjust treatment accordingly [48]. It is necessary to confirm a causative link using larger

multi-center prospective clinical studies. A larger clinical study would allow for analysis of CYP2D6 allelic variations and would improve the causational link between the ultra-rapid metabolizers and Type 2 Diabetes Mellitus treatment. Subsequently, the results of such studies can justify the use of actionable CYP2D6 testing to help better predict diabetic drug response. Furthermore, such clinical studies can be applied to numerous drugs with a narrow therapeutic range to identify patients at risk for drug metabolism inefficiency.

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