# Genetic Variants of the Drug-metabolizing Enzyme CYP2D6 in Puerto Rican Psychiatry Patients: a Preliminary Report and Potential Implications for Breast Cancer Patients

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Background: The CYP2D6 liver enzyme, which metabolizes 25-30% of common medications, is highly polymorphic. Existing studies of Hispanics have focused on Mexicans and Mexican-Americans. The goal of the study was to identify the CYP2D6 alleles associated with reduced or negligible activities present in the Puerto Rican population.

Methods: The study cohort comprised 40 Puerto Rican psychiatric patients referred because of suspected intolerance of drugs metabolized by CYP2D6, and five subjects without suspected adverse responses to these drugs. All subjects had both parents and all grandparents born in Puerto Rico. Genomic DNA was queried for 27 CYP2D6 alleles using the Roche AmpliChip P450 test.

Results: A total of 12 alleles were identified. The most common alleles were CYP2D6*1>*2>*4>*41. The inactive alleles were *4>*5>*31 >*40; reduced activity alleles were *10>*17>*9*=*29; active alleles were *1>*2>*35. Two subjects carried the rare *31 allele. Only one subject carried two non-functional alleles (CYP2D6*5/*40), and was predicted to be a poor metabolizer.

Conclusions: Any conclusions should be interpreted with caution given the small population sample investigated. Nonetheless, our findings strongly suggest that Puerto Ricans exhibit distinct CYP2D6 allele frequencies and harbor a non-functional allele that is rare or absent in other populations and are highly valuable for the emerging practice of Personalized Medicine in admixed populations like Puerto Ricans. [P R Health Sci J 2010;3:299-304]

Key words: AmpliChip, Hispanic, CYP2D6

CYP2D6, a P450 drug-metabolizing enzyme predominantly expressed in the liver, is involved in the metabolism of up to 25% of current medications (1-3), many of which are antidepressants and antipsychotics. Moreover, because CYP2D6 has more than 70 known alleles, it is an important major source of interethnic variability and causes a wide range of activity leading to highly variable drug metabolism. The prevalence of the various CYP2D6 alleles has been studied extensively among the populations of Europe, Asia and Africa (1-3). Nonetheless, relatively little information has been obtained from Central and South America, and none from Caribbean nations.

Four CYP2D6 phenotypes have been recognized concerning their capacity to metabolize these drugs (1-2) these are: 1) ultra-rapid metabolizer, UM, with at least 3 functional gene copies; 2) extensive metabolizer, EM, with normal (wild-type) activity; 3) intermediate metabolizer, IM, with combinations of active and inactive or reduced activity alleles, and 4) poor metabolizer, PM, when both alleles are inactive. The frequency of the EM

phenotype in European Caucasian populations is estimated at 65-80%, IM is 10-15%, PM is 5-10% and UM is 1-5 % (2). The UM phenotype is most prevalent in Ethiopia, 29% (4), and Saudi Arabia, 21% (5), and elevated around the Mediterranean rim, with a 10% prevalence in northern Spain (6). Individuals with the extreme genotypes, PM and UM, are potentially at increased risk for problems with CYP2D6 substrate drugs.

Most antidepressant and antipsychotic drugs are metabolized, at least in part, by CYP2D6. Evidence has been presented that

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persons who are poor metabolizers (PMs) may be at increased risk for dose-related adverse events caused by drugs that are metabolized predominately by CYP2D6, while patients with multiple copies of active genes, UMs, may be more likely to experience therapeutic failure due to rapid metabolism (7). Other drug classes that are predominately metabolized by CYP2D6 include beta blockers like metoprolol, indicated for hypertension (8) and tamoxifen (9), which has been used for more than three decades to reduce recurrences of estrogen-receptor positive breast cancer following surgery (10).

In order to develop pharmacogenetic dosing guidelines tailored towards Puerto Ricans and other admixed Caribbean populations the development of a database collecting pharmacokinetic and pharmacodynamic information is essential. In view of the paucity of CYP2D6 studies among Latinos, we conducted a pilot study geared toward the identification of non-functional and reduced activity CYP2D6 alleles in Puerto Ricans. We employed the AmpliChip microarray, which queries 27 of the CYP2D6 alleles that are most common in populations with European, Asian and African origins (11). Because our pilot study had limited funding, we recruited patients with suspected intolerance of drugs that are metabolized by CYP2D6 for a greater chance to detect non-functional and reduced activity alleles.

## Materials and methods

### Pilot study participants and recruiting strategy

This is a non-interventional cross-sectional pilot study of Puerto Rican psychiatry patients with suspected intolerance of drugs metabolized by CYP2D6. Forty-five Puerto Rican mental health patients were identified by their mental health providers and referred to the study in 2008. All subjects had both parents and all grandparents born in Puerto Rico (information supplied by the patient), and were at least 18 years of age, clinically stable and currently taking drugs that are metabolized wholly or in part by CYP2D6, including antidepressants (n=9), antipsychotics (n=33), and atomoxetine (n=3). Personal and clinical information was obtained from the patients and/or their mental health provider. Although other clinical information was also obtained, (e.g. a questionnaire about adverse events) the results are not relevant to the present report. The study was carried out with the permission of the pertinent government agency and the IRB of the University of Puerto Rico Medical Sciences Campus. Each participant gave written informed consent. The adverse events-strategy was intended to enrich the pool of alleles associated with no or reduced enzyme activity. The strategy was based on a study of patients who had severe adverse events to antidepressant drugs that were metabolized by CYP2D6 (7). In the cited study, the frequency of patients who had two inactive alleles, i.e., poor metabolizers, was 29%, which represented a four-fold increase over the general population.

### Genotyping

Approximately 6 ml of blood were obtained from each enrolled subject and frozen at -80°F. Genotype analysis was carried out by the Esoterix laboratory (Research Triangle, North Carolina) with the Roche AmpliChip CYP450 Test as recommended by the manufacturer (11). The CYP450 microarray detects 27 CYP2D6 alleles, including CYP2D6*2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *15, *17, *19, *20, *29, *35, *36, *40, *41, *1XN, *2XN, *4XN, *10XN, *17XN, *35XN, *41XN. The data analysis software for the AmpliChip CYP450 test analyzes the intensity pattern of each probe cell and determines the genotype at each specified polymorphic site based on the unique pattern of nucleotide changes that define each allele. The defining polymorphisms utilized by the AmpliChip software include SNPs, deletions, insertions and duplication-specific sequences. If no sequence variations are detected, the CYP2D6*1 allele (wild-type) is assigned. The SNP (single nucleotide polymorphism) pattern in two patients could not be interpreted by the AmpliChip CYP450 Test allele algorithm. These samples were subsequently genotyped (12) using a strategy employing long-range PCR (polymerase chain reaction) and PCR-RFLP(restriction fragment length polymorphism)-based SNP detection as described in detail elsewhere by Gaedigk et al. (13). Briefly, the entire CYP2D6 gene is first amplified by long-range PCR to produce a CYP2D6-specific genotyping template which subsequently serves as a template for a series of genotyping reactions identifying the presence of allele-specific 'key' SNPs.

### Calculation of activity score and allele frequencies

A score reflecting the predicted activity (i.e., Activity Score, AS) was assigned to each genotype using the method of Gaedigk et al. (13) as follows: a fully active allele received a value of 1.0, while values of 0.5 and 0 were assigned to alleles associated with reduced or no activity, respectively. The sum of values assigned to both alleles forms the Activity Score. Thus, the predicted AS for a CYP2D6*5/*41 genotype is 0.5, since *5 is a null allele and *41 is associated with reduced activity.

## Results

### Characteristics of patients

The patient cohort comprised 14 male and 31 female psychiatric patients. The average age was 42.9 ± 14.3 years (mean ± standard deviation), and the age range from 20.7 to 89 years. Patients were mainly from the eastern half of the island, although all seven senatorial districts of Puerto Rico were represented when parents' birthplace was considered. Five convenience patients without significant adverse events related to CYP2D6 substrate drugs were also genotyped.

We identified 12 CYP2D6 different alleles in our Puerto Rican patient cohort. The most common alleles in our patient population were CYP2D6*1, *2, *4 and *41. Other alleles, in

order of frequency in all patients, were *CYP2D6*10>*17>*5= *31>*9>*29=*36=*40* (Table 1). Among patients with reported suspected intolerance or adverse events (n=40 participants; 80 alleles), 57.5% (n=46) of the alleles encoded fully functional enzymes; 22.5% (n=18) carried alleles associated with reduced function and 20% (n=16) of the alleles were non-functional. Based on a common method of phenotype predictions (i.e., 2), the frequency of EM in this population sample was 60%, IM was 38%, and PM was 2%.

The genotypes identified in all 45 subjects are shown in Table 2. An activity score was calculated for each genotype according to the method of Gaedigk et al. (13). Twenty-seven subjects (60%) had AS of 1.5 or 2.0, fourteen (31%) had AS of 1.0, three (7%) had AS of 0.5, and one (2%) had AS of 0. The patient predicted to be a PM had a *5/*40 genotype. The patients with an AS of 0.5 and predicted to exhibit significantly reduced CYP2D6 activity had *4/*9, *4/*41 and *9/*31 genotypes. Notably, two patients carried the relatively rare nonfunctional *CYP2D6*40* allele believed to have African origin (14) and two patients were heterozygous for *CYP2D6*31*, another rare, nonfunctional allele, which we have recently also discovered in two Spanish and one Hispanic subjects (12). These two alleles are not included in many commercial genotyping tests.

## Discussion

We identified 12 polymorphic *CYP2D6* alleles in 45 Puerto Rican patients with suspected intolerance of medications –psychotropic drugs- that are metabolized principally by CYP2D6. The most common alleles found were *CYP2D6*1>*2>*4>*41*, the first two have full metabolic activity, while *4 has no activity and *41 has reduced activity. Two patients were found to carry the rare inactive *CYP2D6*31* allele. One patient was found to carry two inactive alleles in an uncommon combination, i.e. *CYP2D6*5/*40*. The non-functional *CYP2D6*3* and *6* allele, which have been detected in Mexican-Americans (15), were not encountered in the Puerto Rican sample. The *CYP2D6*40* and *31* alleles, found in Puerto Ricans, both of which are inactive, have not been reported in Mexicans.

The findings obtained in this Puerto Rican sample can not be extrapolated to a general population given the fact that this was a small sample of a particular population. However, these preliminary data suggest that the *CYP2D6* genetic profile in Puerto Ricans is unique and serves as a window to the variability present in the Puerto Rican population. The tetrad of *CYP2D6*1, *2, *4* and *41* is characteristic of European-derived

**Table 1**. CYP2D6 Allele Frequencies (%)  in Latino, African-American and US Caucasian population samples

| Allele | Activity of allele product | PRa (n=45) | Mexican American (n=264) [15] | US Caucasian (n=206) [16] | US Caucasian (n=347) [12, 13] | African American (n=222) [20] | African American (n=272) [12, 13] | Brazilian [17] Black (n=87) | Brazilian [17] (European) (n=278) |
|---|---|---|---|---|---|---|---|---|---|
| Method | | Ampli Chip | PCR | GeneChip and PCR | PCR | AmpliChip prototype | PCR | MassARRAY SNP genotyping system | |
| *1 | Normal | **37.8** | **55.1** | **39.6** | **36.5** | **35.3** | **32.9** | **41** | **40** |
| *2 | Normal | **18.9** | **18** | **18.7** | **22.9** | 4.5 | **14** | **13** | **16.4** |
| *3 | Non-functional | 0 | 0.2 | 1 | 1 | 0.45 | 0.2 | 1.15 | 0.7 |
| *4 | Non-functional | **12.2** | **10** | **20.9** | **19.7** | **6.5** | 3.9 | 6.2 | **12.2** |
| *5 | Non-functional | 3.3 | 1.7 | 2.7 | 3.3 | 5.6 | 6.4 | 4 | 1.8 |
| *6 | Non-functional | 0 | 0.4 | 0 | 1 | 0.23 | 0.6 | 0 | 1.1 |
| *9 | Reduced | 3.3 | 1.1 | 2.2 | 3 | 0.9 | 0.2 | 0 | 1.8 |
| *10 | Reduced | 4.4 | 2.8 | 0.7 | 2.2 | 3.6 | 2.9 | 4 | 2.3 |
| *17 | Reduced | 3.3 | 0.2 | 0 | 0.4 | **14.9** | **19.1** | **9.2** | **1.6** |
| *29 | Reduced | 1.1 | 0.2 | n/d | 0.1 | 5.2 | **7.5** | **6.3** | 0.2 |
| *31 | Non-functional | 2.2 | n/d | n/d | [0]b | n/d | [0]b | n/d | n/d |
| *35 | Normal | 1.1 | n/d | n/d | n/d | 0.68 | nd | 0 | 6.7 |
| *40 | Non-functional | 2.2 | n/d | n/d | 0 | 0.68 | 0.6 | 0 | 0.36 |
| *41 | Reduced | **10**d | **9.5**c | **10.2**d | **8.1**c | **14.4**d | 1.8c | **10.9**d | **7.91**d |
| All Duplicated | | 0 | 0.8 | 2 | 1.9 | 5.39 | 5.1 | 3.4 | 5.2 |
| % Fully functional | | 57.8 | 73.1 | 58.3 | 72 | 39.8 | 46.9 | 54 | 63.1 |
| % Reduced activity | | 22 | 13.8 | 19.4 | 5.4 | 39 | 35.4c | 31.04 | 13.81 |
| % Inactive | | 19.9 | 12.1 | 24.6 | 24 | 13.46 | 15.6 | 13.2 | 16.16 |

n, number of subjects
n/d, allele not queried
The four most prominent alleles are in bold and underlined
a Subjects were preselected for suspected intolerance of CYP2D6 substrate drugs
b Determined in a random pool of 100 subjects [12]
c Identified by 2988A
d Identified by -1584C, 1661C, 2850T and 4180C

populations in the United States (13, 16). The same four alleles also predominate in Mexican-Americans (15) and European-derived Brazilians (17). Studies performed prior to 2000 do not provide any information for the *41 allele, which codes for a reduced-activity enzyme (18-19) (depending on the extent of genotyping performed, *41 was classified as *1 or *2). The CYP2D6*41 is still often neglected, especially when 'limited' genotyping is performed. The frequency of CYP2D6*41 appears to be variable in Africans and their descendants (13, 20). This may in part be due to identifying the CYP2D6*41 allele via the -1584C>G SNP which is in almost perfect linkage in Caucasians, but not in Africans and their descendants (i.e. only a portion of alleles called CYP2D6*41 carry the 'key' SNP at position 2899 which causes reduced activity) (21). Depending on the population studied, the four most prominent alleles may also include CYP2D6*5, a non-functional allele that is found globally (22). The Puerto Rican sample was highly heterogeneous and was found to have characteristics of both European and African-derived populations, consonant with its immigration history. These findings are also in line with Ruaño et al. (23) who found the population to be broadly heterogeneous after genotyping 100 representative Puerto Rican blood samples. They identified three main gene clusters possibly representing the ancestral European, African and Amerindian populations.

**Table 2.** CYP2D6 genotypes in Puerto Ricans

| Genotype | Frequency (%) | n | Activity Score [13] |
|----------|---------------|---|---------------------|
| *1/*1 | 13.3 | 6 | 2 |
| *1/*2 | 11.1 | 5 | 2 |
| *2/*2 | 2.2 | 1 | 2 |
| *1/*9 | 2.2 | 1 | 1.5 |
| *1/*10 | 4.4 | 2 | 1.5 |
| *1/*17 | 4.4 | 2 | 1.5 |
| *1/*41 | 8.9 | 4 | 1.5 |
| *2/*10 | 2.2 | 1 | 1.5 |
| *2/*17 | 2.2 | 1 | 1.5 |
| *2/*29 | 2.2 | 1 | 1.5 |
| *2/*41 | 6.7 | 3 | 1.5 |
| *1/*4 | 11.1 | 5 | 1 |
| *1/*5 | 2.2 | 1 | 1 |
| *1/*31 | 2.2 | 1 | 1 |
| *1/*40 | 2.2 | 1 | 1 |
| *2/*4 | 6.7 | 3 | 1 |
| *2/*5 | 2.2 | 1 | 1 |
| *4/*35 | 2.2 | 1 | 1 |
| *10/*41 | 2.2 | 1 | 1 |
| *4/*9 | 2.2 | 1 | 0.5 |
| *4/*41 | 2.2 | 1 | 0.5 |
| *9/*31 | 2.2 | 1 | 0.5 |
| *5/*40 | 2.2 | 1 | 0 |

We also discovered two subjects carrying the elusive CYP2D6*31 allele (12). A single instance of this allele was first identified in a study of 672 unrelated European subjects (24)

and subsequently further characterized in vivo and in vitro (12, 25) and with crystal structure modeling (26). The unexpected identification of a rare European allele in the population raises the possibility that other CYP2D6 alleles, as well as alleles of other polymorphic genes, may be more common in Puerto Ricans than other populations. The family birthplace information provided by research subjects suggests that the two subjects are not closely related, but may be members of an extended family.

We did not detect any gene duplication events in this sample, which may be due to the recruiting strategy based on adverse events, or may be a random result due to the small sample size. These same reasons may also explain why we discovered only a single poor metabolizer among the 45 patient cohort. Since the population comprised patients who had recent medication intolerance, it is possible that older poor metabolizers had been switched to medications that were not CYP2D6 substrates early in the course of their treatment, thus depriving the cohort of PMs.

It has been argued that there is *unethical conduct* if an underpowered study is performed in human subjects, because results cannot be generalized and consequently human subjects have been unnecessarily exposed to the risks associated with experimental procedures. However, this was a pilot study to obtain initial data. The only procedure performed with patients in the present study was collection of a single blood sample by venipuncture, a common intervention that involves no more than minimal risk (27). Despite the relatively small data set, the information obtained is highly valuable for the planning of future investigations.

Moreover, we were able to accomplish our primary goal of identifying inactive and reduced activity alleles present in the study cohort employing an adverse-event recruiting strategy which we designed after a study of German psychiatry patients with adverse events related to antidepressants metabolized by CYP2D6 (7). In this study, the authors concluded that genotypic poor metabolizers were four-fold higher in the subpopulation of patients who had experienced adverse events compared to those in the general population. In the present study, we were unable to determine whether allele and genotype frequencies differ between patients and healthy controls, because there are no data available for the Puerto Rican population. Nonetheless, at least half of the 12 alleles identified in our Puerto Rican population sample are present at levels of less than one percent in other populations (Table 1) strongly suggesting that the adverse events strategy was successful in identifying rare alleles that otherwise might not have been present in a random sample of 45 individuals. Our results and those of Ruaño et al. (23) demonstrated that the Puerto Rican population is heterogeneous and harbors many non-functional and reduced activity CYP2D6 alleles.

Although the present descriptive study was conducted with psychiatry patients with suspected drug intolerance we considered that these findings might also have implications for Puerto Rican breast cancer patients. Tamoxifen, which has

been widely used for over 30 years to reduce the recurrence of estrogen-dependent breast cancer (10), is metabolized by CYP2D6. In 2003 Stearns et al (9) reported that tamoxifen is a CYP2D6 pro-drug, and must be activated by metabolism to endoxifen, an estrogen receptor antagonist. Patients who have reduced CYP2D6 activity, i.e., IM or PM, can produce only a small amount of tamoxifen through a minor pathway, and may be at increased risk for recurrences of breast cancer, despite the use of tamoxifen (28-33). Some studies disagree with this conclusion (34-36), although a consensus seems to be forming that active CYP2D6 metabolism is important. Many drugs also inhibit CYP2D6 and reduce the production of endoxifen. The most potent inhibitors include the antidepressants bupropion, fluoxetine and paroxetine, as well as the class Ia antiarrhythmic quinidine and cinacalcet, indicated for secondary hyperparathyroidism. The ability to predict endoxifen plasma levels is greatly improved when both CYP2D6 genotype and the inhibitory potency of co-medications is considered (37).

At present, there is insufficient knowledge to predict the prevalence of Puerto Rican or other Hispanic breast cancer patients who are not able to convert tamoxifen to its active form in an efficient manner. Unfortunately, commercial genotyping assays are still relatively costly, and screening for only the most common alleles may fail to detect certain alleles present in the Puerto Rican population.

## Resumen

Introducción: La enzima hepática CYP2D6, que metaboliza el 25%-30% de los medicamentos mas comunes, es altamente polimórfica. Los estudios existentes de Hispanos han sido con mexicanos y mexicanos viviendo en los Estados Unidos. El objetivo del estudio era identificar los alelos del CYP2D6 asociados con actividad reducida o inactivos presentes en la población de puertorriqueños. Métodos: Los sujetos, todos puertorriqueños, incluyen 40 pacientes psiquiátricos referidos por intolerancia de drogas metabolizadas por CYP2D6 y cinco pacientes sin efectos adversos a estas drogas. Según información provista por los sujetos, todos sus padres y abuelos nacieron en Puerto Rico. El análisis utilizó la Roche AmpliChip P450 Test para examinar el ADN genómico aislado de leucocitos para 27 alelos. Resultados: Se identificaron 12 alelos; los más comunes fueron CYP2D6*1>*2>*4>*41. Los alelos inactivos que se identificaron fueron *5>*31 >*40; mientras que los alelos con actividades reducidas fueron *10 >*17>*9*=*29; y, los alelos activos *1>*2>*35. Dos sujetos tuvieron el alelo *31, el cual es poco frecuente. Solo un sujeto presentó dos alelos no-funcionales (CYP2D6 *5/*40), lo cual predice el fenotipo de "metabolismo lento". Conclusiones: Cualquier conclusión se debe hacer con cautela dado el pequeño tamaño de la muestra que fue examinado. Sin embargo, estos hallazgos sugieren que los puertorriqueños exhiben distintas frecuencias de los alelos de CYP2D6 incluyendo un alelo nulo que es infrecuente o ausente de otras poblaciones y sientan las bases para lo que será la futura práctica de Medicina Personalizada en poblaciones mixtas, tales como los puertorriqueños.

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