The Prevalence of CYP2D6 Gene Polymorphisms among Filipinos and their use as Biomarkers for Lung Cancer Risk

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ABSTRACT

Objectives. The highly polymorphic nature of the CYP2D6 gene and its central role in the metabolism of commonly used drugs make it an ideal candidate for pharmacogenetic screening. This study aims to determine the prevalence of CYP2D6 polymorphisms among Filipinos and their association to lung cancer.

Method. Forty seven single nucleotide polymorphisms (SNPs) of the CYP2D6 gene were genotyped from DNA samples of 115 cases with lung cancer and age- and sex-matched 115 controls.

Results. Results show that 18 out of 47 polymorphisms have significant genotypic variability (>1% for at least 2 genotypes). No variant is associated with lung cancer. However, rs1135840, rs16947 and rs28360521, were found to be highly variable among Filipinos.

Conclusion. This study demonstrated that CYP2D6 polymorphisms are present among Filipinos, which, although not found to be associated with lung cancer, can be useful biomarkers for future pharmacogenetic studies. The SNP rs16947 is found to be associated with cancer and timolol-induced bradycardia; the SNP rs1135840, on the other hand, is only shown to be linked with cancer. The genetic variant rs28360521 is known to be associated with low-dose aspirin-induced lower gastrointestinal bleeding.

Key Words: Filipino, pharmacogenetics, CYP2D6, lung cancer, biomarker, SNP

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Introduction

Cytochrome P450 2D6 (CYP2D6), a member of the cytochrome P450 oxidase system, is one of the most studied enzymes in the metabolism of various substrates in the human body. It codes for debrisoquine hydroxylase, which comprises only about 1-5% of human hepatic CYP450 enzymes. Despite this, about 25% of known drugs and

xenobiotics are metabolized by CYP2D6, and several endogenous compounds such as hydroxytryptamines and neurosteroids are also metabolized by the enzyme.[1] In addition, the CYP2D6 gene locus is located in the short arm of chromosome 22, and is highly polymorphic, with at least 100 alleles which vary in distribution among and within diverse ethnic and ancestral populations.[2,3] This leads to substantial phenotypic variations among different individuals and populations, and it has become a central target for pharmacogenetic studies. Hence, with its metabolic impact and the wide inter-individual variations, genetic testing for CYP2D6 variations offers the possibility of clinical applications predicting those who may likely benefit from a specified substrate of CYP2D6 and more importantly, those at-risk for a xenobiotic-induced adverse effect.

The significant difference in the activity of CYP2D6 among individuals correlates well with variations in the CY2D6 alleles in the human genome. Depending on the genomic variant, a considerable range of functional efficiency and expression of the enzyme is observed.[3,4,5] The variants are classified depending on the functional capacity of the enzyme: non-functional (such as *3, *4 and *5), reduced functional (such as *9, *10, *17, *29 and *41), and functional (such as *1/wild type, *2A *35 and *39). Phenotypically, an individual with non-functional alleles only is a poor metabolizer (PM); with 1 or 2 reduced functional and non-functional allele as intermediate metabolizer (IM); and with 1 or 2 functional alleles as extensive metabolizer (EM). In addition, the CYP2D6 gene may have genomic duplications resulting to amplified functional activity (3 or more alleles) that result to ultrarapid metabolizer (UM) phenotype.[4] PMs will predictably result to higher blood levels of a particular CYP2D6 substrate and reduced levels of the corresponding metabolites, and EMs and UMs have lower levels of the substrates and higher levels of their corresponding metabolites. The variation in terms of functional capacity is very significant that inter-individual metabolic rates may differ by more than 10,000-fold.[5] Because of its medical utility in drug metabolism, practice guidelines on the CYP2D6 gene were formulated for particular drugs by The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG).[3]

The prevalence of variations in CYP2D6 has marked differences among different ethnic groups. The non-functional haplotype CYP2D6*4 is the most common cause of reduced activity in Caucasians with an allele frequency of 0.207. Other variations, CYP2D6*2A, CYP2D6*3, CYP2D6*5, CYP2D6*6, and CYP2C9*2 were also present in this population with allele frequencies of 0.324, 0.020, 0.020, 0.009, and 0.16, respectively.[5,6,7] In Africans, CYP2D6*2 and CYP2D6*17 are more common. In contrast, the haplotypes are rare in Asians, and instead, the reduced functional haplotype CYP2D6*10 (with a prevalence of 51.3% among mainland Chinese and 41.17% among Hong Kong Chinese) is present in about half of Asians.[2,5,8] Moreover, a higher prevalence of UM is observed in Caucasians at about 7%. In general, PMs (5-10%) and UMs (about 7%) are more common among Caucasian, and IMs are more common among Asians (about 50%).[1] It was found out that among 70-90% of all PMs, the CYP2D6*4 allele is prevalent.[7]

CYP2D6 genotyping is gaining interest in pharmacogenetics. The clinical significance of the CYP2D6 variants is on their marked functional effect on the enzyme resulting to trait differences. Studies demonstrate that certain haplotypes may be useful to predict patients that may not respond to tamoxifen therapy in breast cancer. Because CYP2D6 converts tamoxifen to its biologically more active metabolite endoxifen, PMs and IMs do not respond well to tamoxifen treatment. This results to lack of clinical response, the need for higher dosages, or disease relapse.[9-12] To demonstrate the potential use of CYP2D6 genotyping on tamoxifen use, one research explored the clinical utility of CYP2D*4 screening. In their meta-analyses, the prevalence of CYP2D6*4 is 27% in Caucasians.[13] More importantly, the prevalence of CYP2D6*4 is 90% in a subset of PMs. This has led to several studies looking into the functionality of such screening in the clinical setting. However, the clinical relevance of CYP2D6*4 screening is hampered by conflicting results in studies using clinical outcomes, such as disease-free duration and survival. In another large meta-analysis study, Province et al. suggested that a more restrictive criterion incorporating clinical parameters may benefit more from CYP2D6 genotyping. In their study, postmenopausal women with estrogen receptor-positive breast cancer, receiving 20 mg/day tamoxifen for 5 years benefitted better than other subjects.[10] Factors such as treatment compliance and inhibitors of the CYP2D6 enzyme combined with tamoxifen may have an effect on the results of studies on the association of CYP2D6 and tamoxifen therapy.[11] Nevertheless, genotyping of the CYP2D6 gene is still commonly done for individualized therapy of tamoxifen for patients with breast cancer.[12]

Another possible pharmacogenetic use is in relation to psychotropic agents. Other drugs that have strong evidence to correlate with the CYP2D6 phenotypes include antipsychotics such as haloperidol and risperidone, opioids such as codeine and tramadol, and antiarrhytmics such as metoproplol and propafenone.[1] For Asians, the most common CYP2D6 SNP, CYP2D6*10, has been associated with rates of metabolism of commonly used drugs, such as metoproplol.[14]

Besides the potential utility of CYP2D6 in drug administration and adverse events, another importance of the gene is its possible involvement in disease susceptibility. CYP2D6 has been implicated in carcinogenesis, particularly of head and neck cancers, lung cancer and breast cancer.[5,8,15] Lung cancer continues to be one of the most common causes

of cancer deaths worldwide.[16] In the Philippines, it ranks among the top three causes of mortality among cancer patients.[17] Survival from lung cancer is poor (5-10% at 5 years) and to date, no effective screening for an at-risk individual is available. Previous genetic studies presented evidence that occupational exposure, diet, smoking history, and presence of lung cancer in the first-degree relatives pose risk for lung cancer.[18,19] A growing interest is on the possibility of incorporating genetic factors to clinical parameters in fine tuning medical aids in the prediction of disease occurrence.

Specifically for lung cancer, studies show that CYP2D6 is responsible for converting tobacco-derived substrates to potential lung carcinogens such as polyaromatic hydrocarbons (PAH) that may form adducts to DNA and proteins, and the nicotine metabolite cotinine.[5,20] Some studies show an association of cancer to CYP2D6 variants, including lung cancer.[8,14] However, in general, the data on the association of CYP2D6 and cancer is inconclusive and at best, only limited to a few small studies.[8]

Methods

Subject Population and Sample Collection

This case/control study was approved by the University of the Philippines Manila Ethical Review Board (UPM REB). The study included 115 lung cancer patients and 115 controls. The case group was composed of males and females ranging from 20 to 87 years old (mean = 52.32) from four hospitals in Metro Manila, namely, Philippine General Hospital, Jose R. Reyes Memorial Medical Center, Medical Center Manila, and Lung Center of the Philippines. The control group was composed of males and females ranging from 15 to 89 years old (mean = 51.96). The controls were recruited from the general clinics (patients and their companions) of the same hospitals. The controls were matched by age (±5 years) and sex to the lung cancer cases. Informed written consents were obtained from all subjects before recruitment and blood extraction.

DNA Extraction and Quantification

DNA was extracted from blood samples by using the QIAamp DNA minikit for blood and body fluids in accordance with the manufacturer's instructions. DNA samples were stored in -20°C after extraction. They were then diluted to a concentration of 50 ng/uL prior to microarray processing.

SNP Genotyping

Genomewide scan of human DNA samples was done using DNA microarray technology, specifically the Illumina HiScan system, following the GoldenGate Genotyping (GGGT) assay protocol specified in the manufacturer's manual. Initially, screening for single nucleotide polymorphisms (SNPs) among genes clinically associated with lung cancer was done using genetic databases. A list of SNPs with the probes was completed based on dbSNP database version 131. The identified SNPs were then submitted to Illumina for scoring and the SNP list was finalized based on their scores prior to processing of bead chips by Illumina.

Data Analysis

The cases and the corresponding controls were age-matched and sex-matched and have a 1:1 case/control ratio. GoldenGate genotyping data was analyzed using GenomeStudio. Samples that have poor performance in the genotyping assay were excluded from the analysis. SNP clusters were evaluated and edited to ensure high quality data. SNPs with overlapping clusters and low call frequencies were zeroed. The genotype frequencies were calculated and the data on zygosity of SNPs were established. Statistical analyses were performed using R ver2.15.1. A logistic regression analysis for genotype status was performed. Comparison of characteristics between groups was carried out through Fisher's exact tests for genetic variables. A p-value <0.05 was deemed statistically significant.

Results and Discussion

To determine the prevalence of CYP2D6 polymorphisms in the Filipino population, 47 CYP2D6 SNPs were genotyped for the case-control analysis. After the genotyped SNPs were evaluated and edited, only 38 SNPs out of 47 were included in the analysis. A couple of SNPs had overlapping clusters and were excluded. Some had low call frequencies and were zeroed. The call frequency is equal to the number of samples receiving a genotype call divided by the total number of samples. A summary of the 38 SNPs and their description is presented in Table 1. There are 2 SNPs with call frequencies that are less than 1, rs28566059 and rs28371738, as one sample failed to be genotyped for each aforementioned SNP.

Out of the 38 SNPs used in the study, there are 18 CYP2D6 SNPs with significant genotypic variability within the study population (Table 2). With frequencies of more than 1% in at least 2 of the AA, AB, and BB genotypes, these SNPs can be considered as polymorphic among Filipinos. One SNP, rs28641480, has a prevalence of 0.87% that did not reach significance. Nevertheless, such borderline number may be further verified in a larger population. This may provide insight into the genetic variability of Filipinos as regards the CYP2D6 gene, further supporting the notion of the existence of a unique genetic profile of the population compared with other ethnicities. The other 17 CYP2D6 SNPs are exclusively homozygous to either allele A or B, suggesting that Filipinos may have unique variations that may affect their phenotypes.

Table 1. List of CYP2D6 SNPs included in the study*

SNP	Position in Chr22	Allele (A/B)	Potential function†	Code change	Predicted effect‡	Minor allele frequency		
						African	Asian	Caucasian
rs28579115	42530826	T/G	Transcription factor binding site		--	--	--	--
rs28641480	42530640	A/G	Transcription factor binding site		--	--	--	--
rs62239767	42530507	C/G	Transcription factor binding site		--	--	--	--
rs74817548	42530423	A/G	--		--	--	--	--
rs78861686	42530327	A/G	--		--	--	--	--
rs28842514	42529930	T/G	Transcription factor binding site		--	--	--	--
rs28542726	42529407	T/G	Transcription factor binding site		--	--	--	--
rs28566059	42529321	T/C	Transcription factor binding site		--	--	--	--
rs28597993	42529256	T/G	Transcription factor binding site		--	--	--	--
rs73887946	42529156	T/C	--		--	--	--	--
rs28360521	42528976	T/C	Transcription factor binding site		--	--	--	--
rs75112600	42528751	A/G	--		--	--	--	--
rs1080983	42528568	A/G	Transcription factor binding site		--	--	--	--
rs28588594	42528224	A/G	Transcription factor binding site		--	--	--	--
rs35046171	42527065	T/C	Transcription factor binding site		--	--	--	--
rs28371694	42526890	T/C	Transcription factor binding site		--	--	--	--
rs72549358	42526775	T/C	--		--	--	--	--
rs28371699	42526484	T/G	Transcription factor binding site		--	--	--	--
rs71328650	42525952	A/C	--		--	--	--	--
rs28439001	42525651	A/G	--		--	--	--	--
rs79931073	42525501	C/G	--		--	--	--	--
rs67497403	42525280	A/G	--		--	--	--	--
rs72549355	42524850	T/C	--		--	--	--	--
rs58440431	42524696	T/C	--		--	--	--	--
rs75203276	42524501	A/G	--		--	--	--	--
rs28371717	42524310	T/G	Non-synonymous code change; Splice site effect	A237S	Benign	--	--	--
rs28371723	42524033	A/T	--		--	--	--	--
rs16947	42523943	A/G	Non-synonymous code change; Splice site effect	R296C	Benign			
rs79489631	42523547	A/G	--		--	--	--	--
rs75386357	42523475	A/G	--		--	--	--	--
rs78139609	42523386	A/C	--		--	--	--	--
rs79596243	42523315	A/T	--		--	--	--	--
rs915947	42523241	A/G	--		--	--	--	--
rs1135840	42522613	C/G	Non-synonymous code change	S486T	Benign	--	--	--
rs28371738	42522392	T/C	miRNA binding site		--	--	--	--
rs12169962	42522312	T/C	--		--	0.92	0.839	0.695
rs77827855	42522079	T/C	--		--	--	--	--
rs4078247	42521985	T/C	--		--	--	--	--

* Based on dbSNP version 131

† Based on NIEHS SNP Function Prediction Result (http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi)

‡ Polyphen prediction (as based in NIEHS SNP Function Prediction Result)

Interestingly, because of the significantly variable CYP2D6 genotypes in the Filipino samples, the study revealed some potentially useful polymorphisms for clinical applications. One noteworthy polymorphic variant is rs16947. It is a commonly occurring variant worldwide, recently known to be easily predicted phenotypically by CYP2D6 enhancer variants.[3] It was found to be linked with poor metabolizers because of its decreased enzymatic activity.[21] The G carrier of CYP2D6 rs16947 was previously associated with lesser risk for breast cancer.[8] Furthermore, the AA genotype of rs16947 was associated with serious bradycardia induced by timolol, a common treatment for glaucoma.[22] This could be a useful genetic screen for patients taking timolol-containing ophthalmic drops. However, it is noteworthy that even though all Filipinos in the study have the G allele (AG 27.4%, GG 72.6), the genotype distribution seems to be biased as no AA genotype is observed. Seemingly, this possible non-conformance to the Hardy-Weinberg equilibrium needs to be verified (Table 3). Our speculation is that the AA genotype is present in the population, and this should be an interesting topic for future research.

Another remarkable finding is the rs28360521 polymorphism. The importance of this SNP is not obvious as it is predicted to be an intron variant without further significance. However, the SNP has been recently found to be highly associated with the occurrence of clinically significant small bowel bleeding among people taking low-dose aspirin (GG genotype with OR 4.11, 95%CI: 1.62-10.4).[23] Also, the incidence of lower gastrointestinal bleeding on low dose-aspirin is about 4% in Japanese patients. In a pharmacogenomics study using exome sequencing, rs28360521 showed no considerable difference among East Asians, and was significantly different from European populations.[24] In our study population, the GG phenotype has a high estimated prevalence of 22.6%. Since

Table 2. Genotype frequencies of CYP2D6 SNPS included in the study

	SNP ID	AA Freq	AB Freq	BB Freq
1	rs1080983	0.01	0.26	0.73
2	rs28566059	0.27	0.50	0.23
3	rs1135840	0.48	0.38	0.14
4	rs67497403			1.00
5	rs28842514		0.03	0.97
6	rs28641480		0.01	0.99
7	rs28360521	0.27	0.50	0.23
8	rs28371723			1.00
9	rs4078247	0.20	0.51	0.29
10	rs62239767	1.00		
11	rs28579115	0.14	0.39	0.47
12	rs28371738	0.25	0.49	0.25
13	rs28439001			1.00
14	rs75112600	1.00		
15	rs28542726	0.14	0.38	0.48
16	rs79931073			1.00
17	rs78139609			1.00
18	rs71328650	0.12	0.41	0.47
19	rs79489631			1.00
20	rs75203276			1.00
21	rs28371717			1.00
22	rs73887946			1.00
23	rs12169962		0.27	0.73
24	rs78861686			1.00
25	rs28597993			1.00
26	rs74817548			1.00
27	rs28371699	0.47	0.39	0.13
28	rs77827855		0.04	0.96
29	rs72549358			1.00
30	rs28588594	0.21	0.58	0.21
31	rs72549355			1.00
32	rs58440431	0.21	0.51	0.28
33	rs915947		0.22	0.78
34	rs35046171			1.00
35	rs75386357			1.00
36	rs79596243			1.00
37	rs16947		0.27	0.73
38	rs28371694			1.00

low-dose aspirin is a common medical regimen for cardiovascular diseases, the impact of screening for at-risk patients can be high and a screening test can be cost-effective. It is speculated that the site of the variant may have regulatory significance or it can be involved in the splicing process. Moreover, as the cited study failed to address, it would be interesting to determine the association of rs28360521 with low dose aspirin-associated upper GI bleeding.

It is of note that the study has not been able to determine the prevalence of CYP2D6*10 due to technical optimization of the microarray chip. Fortunately, a recent study has reported the prevalence of CYP2D6*10 in Filipinos.[25] The frequency of the reduced functional T allele is 54%, and proportion of the T/T and C/T genotype is 24% and 56%, respectively. For lung cancer, several studies have been inconclusive on its association with the allele.[8] By investigating more novel SNPs in the CYP2D6 loci, the study provided novel insights in the alternative utility of these SNPs in clinical practice.

To determine the association of genotypes to lung cancer, logarithmic regression using different statistical models (additive, dominant, and recessive) was attempted. The genotypic frequencies per SNP for cases and control are presented in Table 4. Only genotypes with at least 1% prevalence in at least 2 combinations are assessed. The odds ratio (OR) values for each CYP2D6 SNP assuming the dominant or recessive model were calculated, and presented in Table 5. Table 6 shows the OR values of 16 CYP2D6 SNPs using the additive genotype model. Results indicate that the genotype distributions of the SNPs were not considerably different from the lung cancer case and control groups, and no significant association of any CYP2D6 variant to lung cancer can be considered.

The results of the study are consistent with most of the studies that show the lack of association of CYP2D6 SNPs with cancer, including lung tumors. In a study involving African Americans and Caucasians, CYP2D6*4, CYP2D6*3, CYP2D6*5 and CYP2D6*16 were not found to be associated to lung cancer.[7,26] Another study investigating CYP2D6*4, CYP2D6*3, CYP2D6*5 and CYP2D6*6A on Americans did not show association (Shaw et al. 1998). In studies involving Han Chinese, no CYP2D6 polymorphism was found to be associated with lung cancer.[15,27] In another study that involves screening of various metabolic marker candidates, CYP2D6 variants did not correlate with the occurrence of lung cancer.[28] This was also verified by a more recent study on metabolic enzymes stating that the CY2D6 gene plays a minimal role in procarcinogen activation.[29] It has thus been speculated that as a metabolic processor of tobacco-derived substrate, CYP2D6 might be a minor player compared to CYP2A6.[20]

In contrast to SNPs, a growing interest is gaining on the role of CYP2D6 duplications on lung oncogenesis. Seemingly, several studies have shown that the effect of copy number variations of functional CYP2D6 alleles on lung cancer is more evident. Compared with inactivating alleles, the presence of gene duplicates was found to be more represented in a sample of African Americans with lung cancer, as well as with the occurrence of lung adenocarcinoma.[26] In another study, such duplications are associated with both lung cancer and cancer of the larynx.[30]

Unfortunately, the microarray technology is limited in detecting targeted duplication; a customized chip can only interrogate a few genes at a time. A microarray-based comparative genomic hybridization approach may be effective, but is costly and may be less feasible for clinical application. Some more cost-effective approaches and routinely applied methods for detection of CYP2D6 copy number variants include restriction fragment length polymorphism (RFLP) assays and PCR-based sequencing assays.[12,31] A developing molecular method such as digital PCR, which uses circulating nucleic acids for biomarker detection, can also be considered when doing variant-based

Table 3. Hardy-Weinberg equilibrium calculation for 2 alleles

	SNP ID	Expected			Allele Frequency		
		AA	AB	BB	A	B	*p value*
1	rs1080983	1.96	24.08	73.96	28 (14%)	172 (86%)	0.4253
2	rs28566059	27.04	49.92	23.04	104 (52%)	96 (48%)	0.9872
3	rs1135840	44.89	44.22	10.89	134 (67%)	66 (33%)	0.1595
4	rs67497403			100		200 (100%)	0.2165
5	rs28842514		2.96	97.02	3 (1.5%)	197 (98.5%)	0.879
6	rs28641480		1	99	1 (0.5%)	199 (99.5%)	0.9599
7	rs28360521	27.04	49.92	23.04	104 (52%)	96 (48%)	0.9872
8	rs28371723			100		200 (100%)	0.2165
9	rs4078247	20.7	49.6	29.7	91 (45.5%)	109 (54.5%)	0.777
10	rs62239767	100		0	200 (100%)		0
11	rs28579115	11.22	44.56	44.22	67 (33.5%)	133 (66.5%)	0.2125
12	rs28371738	24.75	49.5	24.75	99 (50%)	99 (50%)	0.9199
13	rs28439001			100		200 (100%)	0.2165
14	rs75112600	100		0	200 (100%)		0
15	rs28542726	10.89	44.22	44.89	66 (33%)	134 (67%)	0.1595
16	rs79931073			100		200 (100%)	0.2165
17	rs78139609			100		200 (100%)	0.2165
18	rs71328650	10.56	43.88	45.56	65 (32.5%)	135 (67.5%)	0.5123
19	rs79489631			100		200 (100%)	0.2165
20	rs75203276			100		200 (100%)	0.2165
21	rs28371717			100		200 (100%)	0.2165
22	rs73887946			100		200 (100%)	0.2165
23	rs12169962	1.82	23.36	74.82	27 (13.5%)	173 (86.5%)	0.1186
24	rs78861686			100		200 (100%)	0.2165
25	rs28597993			100		200 (100%)	0.2165
26	rs74817548			100		200 (100%)	0.2165
27	rs28371699	44.67	43.66	10.67	133 (67.17%)	65 (32.83%)	0.2881
28	rs77827855		3.92	96.04	4 (2%)	196 (98%)	0.8383
29	rs72549358			100		200 (100%)	0.2165
30	rs28588594	25	50	25	100 (50%)	100 (50%)	0.1096
31	rs72549355			100		200 (100%)	0.2165
32	rs58440431	21.41	50.18	29.41	93 (46.04%)	109 (53.96%)	0.8701
33	rs915947	1.21	19.58	79.21	22 (11%)	178 (89%)	0.2165
34	rs35046171			100		200 (100%)	0.2165
35	rs75386357			100		200 (100%)	0.2165
36	rs79596243			100		200 (100%)	0.2165
37	rs16947	1.82	23.36	74.82	27 (13.5%)	173 (86.5%)	0.1186
38	rs28371694			100		200 (100%)	0.2165

*A p-value <0.05 was deemed statistically significant.

researches.[12] In another perspective, because Asians are rarely UMs compared with the Caucasians, such testing for copy number variants may not be as important. However, a genome-wide genotyping study using 674,518 SNPs of the Affymetrix EUR array showed that a modest amount of European genetic ancestry was detected among self-identified Filipinos in the study. This may warrant further research on the prevalence of UMs among Filipinos.[32]

Nevertheless, some studies suggest an association of CYP2D6 variants to lung cancer. In recent meta-analyses, the CYP2D6 rs1135840 CG, which is present in 37.8% of the subjects of the study, was the only SNP found to be associated with lung cancer (OR 1.92, CI: 1.14-3.21).[8] This is important because this study has been done among Chinese, which have a significant admixture among the Filipino population. This particular SNP was also recently identified to be assisted by CYP2D6 enhancer variants when doing phenotype prediction and expression.[3] It will be interesting to do further investigation on the association of this particular variant using a larger sample size.

Conclusion

This study indicates that out of the 47 SNPs of the CYP2D6 gene investigated, there are 18 CYP2D6 SNPs with significant genotypic variability (>1% for at least 2 genotypes) within the Filipino population. The CYP2D6 SNPs, rs16947, rs1135840 and rs28360521, were found to be highly variable among Filipinos. These SNPs are clinically significant as rs16947 is found to be associated with cancer and timolol-induced bradycardia, while rs1135840, on the other hand, is only shown to be linked with cancer. The SNP rs28360521 is known to be associated with low-dose aspirin-induced lower gastrointestinal bleeding. There are no CYP2D6 polymorphisms found to be associated with the development of lung cancer. The results of the study are consistent with most of the studies that show the lack of association of CYP2D6 SNPs with lung cancer. However, rs1135840, rs16947, and rs28360521 may have clinical implications that can be further studied with a larger sample size. This study demonstrates that the CYP2D6 polymorphisms, although not associated with lung cancer

Table 4. Genotype frequencies of CYP2D6 SNPS among cases and controls

SNP ID	Cases			Controls		
	AA Freq	AB Freq	BB Freq	AA Freq	AB Freq	BB Freq
re1080983	0.03	0.26	0.71		0.26	0.74
re28566059	0.27	0.52	0.21	0.27	0.48	0.25
rs1135840	0.5	0.37	0.13	0.46	0.38	0.16
rs28842514		0.03	0.97		0.02	0.98
rs28360521	0.27	0.52	0.21	0.27	0.49	0.24
rs4078247	0.18	0.53	0.29	0.23	0.49	0.29
rs28579115	0.12	0.37	0.5	0.16	0.4	0.44
rs28371738	0.22	0.54	0.24	0.29	0.44	0.27
rs28542726	0.12	0.37	0.5	0.16	0.38	0.46
rs71328650	0.1	0.43	0.47	0.14	0.38	0.48
rs12169962		0.29	0.71		0.26	0.74
rs28371699	0.5	0.38	0.11	0.44	0.4	0.16
rs77827855		0.07	0.93		0.01	0.99
rs28588594	0.18	0.63	0.19	0.24	0.53	0.23
rs58440431	0.19	0.53	0.28	0.23	0.49	0.29
rs915947		0.19	0.81		0.24	0.76
rs75386357		0.01	0.99			1
rs16947		0.29	0.71		0.26	0.74

Table 5. Odds ratios of CYP2D6 SNPS using the dominant or recessive models

SNP ID	Dominant Model			Recessive Model		
	OR (AB & BB)	95% CI	*p* value*	OR (BB)	95% CI	*p* value*
rs28579115	0.75	0.35 - 1.58	0.57	0.78	0.47 - 1.31	0.43
rs71328650	0.65	0.28- 1.47	0.41	1.04	0.62 - 1.74	1.00
rs28641480	-	-		-	-	0.50
rs28360521	1.00	0.58 - 1.79	1.00	1.22	0.66 - 2.28	0.64
rs12169962	-			1.14	0.64 - 2.04	0.77
rs28542726	0.75	0.35 - 1.58	0.57	0.84	0.50 - 1.41	0.60
rs28371738	0.70	0.38 - 1.27	0.29	1.19	0.66 - 2.17	0.65
rs915947	-			0.74	0.39 - 1.38	0.42
rs28588594	0.69	0.36 - 1.31	0.33	1.23	0.65 - 2.35	0.63
rs58440431	0.81	0.43 - 1.53	0.63	1.04	0.59 - 1.86	1.00
rs28566059	0.99	0.55 - 1.77	1.00	1.23	0.66 - 2.31	0.53
rs28842514	-	-		2.04	0.39 - 14.90	0.68
rs1135840	1.15	0.69 - 1.93	0.69	1.24	0.59 - 2.62	0.71
rs28371699	1.28	0.76 - 2.15	0.43	1.46	0.68 - 3.19	0.44
rs4078247	0.76	0.40 - 1.45	0.51	1.00	0.56 - 1.77	1.00
rs16947	-	-		1.14	0.64 - 2.04	0.77

* Two-sided p value for Fisher's exact test. A p-value <0.05 was deemed statistically significant.

Table 6. Odds ratios of CYP2D6 SNPS using the additive model

SNP ID	AB Genotype		BB Genotype		*p* value*
	OR	95% CI	OR	95% CI	
rs28579115	0.83	0.36 - 1.87	0.68	0.31 - 1.51	0.597
rs71328650	0.61	0.25 - 1.43	0.70	0.29 - 1.63	0.52
rs28641480	-	-	-	-	0.498
rs28360521	0.93	0.50 - 1.73	1.17	0.56 - 2.45	0.791
rs12169962	0.88	0.49 - 1.57	-	-	0.768
rs28542726	0.80	0.35 - 1.79	0.71	0.32 - 1.56	0.709
rs28371738	0.62	0.33 - 1.18	0.87	0.42 - 1.81	0.29
rs915947	1.36	0.73 - 2.57	-	-	0.424
rs28588594	0.64	0.33 - 1.23	0.89	0.40 - 1.98	0.335
rs58440431	0.78	0.39 - 1.52	0.87	0.41 - 1.84	0.753
rs28566059	0.92	0.49 - 1.70	1.17	0.56 - 2.45	0.778
rs28842514	0.49	0.07 - 2.57	-	-	0.683
rs1135840	1.10	0.63 - 1.93	1.29	0.59 - 2.85	0.816
rs28371699	1.19	0.68 - 2.08	1.57	0.71 - 3.59	0.517
rs4078247	0.74	-	0.81	0.38 - 1.71	0.728
rs16947	0.88	0.49 - 1.57	-	-	0.768

*Two-sided p-value for Fisher's exact test. A p-value <0.05 was deemed statistically significant.

among Filipinos, can be useful for future drug metabolism and pharmacogenetic studies.

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Statement of Authorship

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References

1. Samer CF, Lorenzini KI, Rollason V, Daali Y, Desmeules JA. Applications of CYP450 testing in the clinical setting. Mol Diagn Ther. 2013; 17(3):165-84.
2. Teh LK, Bertilsson L. Pharmacogenomics of CYP2D6: molecular genetics, interethnic differences and clinical importance. Drug Metab Pharmacokinet. 2012; 27(1):55-67. Epub 2011 Dec 20.
3. Yang Y, Botton MR, Scott ER, Scott SA. Sequencing the CYP2D6 gene: from variant allele discovery to clinical pharmacogenetic testing. Pharmacogenomics. 2017; 18(7):673-85. doi: 10.2217/pgs-2017-0033. Epub 2017 May 4.
4. Friedrich DC, Genro JP, Sortica VA, et al. Distribution of CYP2D6 alleles and phenotypes in the Brazilian population. PLoS One. 2014; 9(10): e110691. doi: 10.1371/journal.pone.0110691.
5. Khlifi R, Messaoud O, Rebai A, Hamza-Chaffai A. Polymorphisms in the human cytochrome P450 and arylamine N-acetyltransferase: susceptibility to head and neck cancers. Biomed Res Int. 2013; 2013:582768. doi: 10.1155/2013/582768. Epub 2013 Sep 16.
6. Sachse C, Brockmöller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet. 1997; 60(2):284-95.
7. Mota P, Silva HC, Soares MJ, et al. Genetic polymorphisms of phase I and phase II metabolic enzymes as modulators of lung cancer susceptibility. J Cancer Res Clin Oncol. 2015; 141(5):851-60. doi: 10.1007/s00432-014-1868-z. Epub 2014 Nov 12.
8. Zhou LP, Luan H, Dong XH, Jin GJ, Man DL, Shang H. Genetic variants of CYP2D6 gene and cancer risk: A HuGE systematic review and meta-analysis. Asian Pac J Cancer Prev. 2012; 13(7):3165-72.
9. Lim JS, Chen XA, Singh O, et al. Impact of CYP2D6, CYP3A5, CYP2C9 and CYP2C19 polymorphisms on tamoxifen pharmacokinetics in Asian breast cancer patients. Br J Clin Pharmacol. 2011; 71(5):737-50. doi: 10.1111/j.1365-2125.2011.03905. x.
10. Province MA, Goetz MP, Brauch H, et al. International Tamoxifen Pharmacogenomics Consortium. CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. Clin Pharmacol Ther. 2014; 95(2):216-27. doi: 10.1038/clpt.2013.186. Epub 2013 Sep 23.
11. Del Re M, Rofi E, Citi V, Fidilio L, Danesi R. Should CYP2D6 be genotyped when treating with tamoxifen? Pharmacogenomics. 2016; 17(18):1967-69.
12. Cavic M, Krivokuca A, Boljevic I, et al. Pharmacogenetics in cancer therapy - 8 years of experience at the Institute for Oncology and Radiology of Serbia. J BUON. 2016; 21(5):1287-95.
13. Markopoulos C, Kykalos S, Mantas D. Impact of CYP2D*6 in the adjuvant treatment of breast cancer patients with tamoxifen. World J Clin Oncol. 2014; 5(3):374-81. doi:10.5306/wjco.v5.i3.374.
14. Huang Y, Liu X, Kuang X, Liao D. CYP2D6 T188C variant is associated with lung cancer risk in the Chinese population. Tumour Biol. 2013; 34(4):2189-93. doi: 10.1007/s13277-013-0755-6.
15. Khedhaier A, Hassen E, Bouaouina N, Gabbouj S, Ahmed SB, Chouchane L. Implication of Xenobiotic Metabolizing Enzyme gene (CYP2E1, CYP2C19, CYP2D6, mEH and NAT2) polymorphisms in breast carcinoma. BMC Cancer. 2008; 8:109. doi: 10.1186/1471-2407-8-109.
16. Boyle P, Levin B. World Cancer Report 2008.
17. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10. Lyon, France: International Agency for Research on Cancer [Online]. 2010 [cited 2010 Jan]. Available from http://globocan.iarc.fr
18. Ngelangel CA, Javelosa MU, Cutiongco-de la Paz EM, The Philippine Cancer Genetics Study Group. Epidemiological risk factors for cancers of the lung, breast, colon-rectum and oral cavity: a case-control study in the Philippines. Acta Med Philipp. 2009; 43(4):29-34.
19. Nitadori J, Inoue M, Iwasaki M, et al. Association between lung cancer incidence and family history of lung cancer: data from a large-scale population-based cohort study, the JPHC study. Chest. 2006; 130(4):968-75.
20. Caporaso NE, Lerman C, Audrain J, et al. Nicotine metabolism and CYP2D6 phenotype in smokers. Cancer Epidemiol Biomarkers Prev. 2001; 10(3):261-3.
21. López-García MA, Feria-Romero IA, Serrano H, et al. Influence of genetic variants of CYP2D6, CYP2C9, CYP2C19 and CYP3A4 on antiepileptic drug metabolism in pediatric patients with refractory epilepsy. Pharmacol Rep. 2017; 69(3):504-11. doi: 10.1016/j.pharep.2017.01.007.
22. Yan DB, Gill N. Evaluation of 24-hour post-dose efficacy of Travoprost-Timolol and Latanoprost-Timolol fixed-combinations in patients with open-angle glaucoma. Invest Ophthalmol Vis Sci. 2010; 51(13):163.
23. Shiotani A, Murao T, Fujita Y, et al. Novel single nucleotide polymorphism markers for low dose aspirin-associated small bowel bleeding. PLoS One. 2013; 8(12): e84244. doi:10.1371/journal.pone.0084244.
24. Yang L, Lu YL, Wang HJ, Zhou WH. [Pharmacogenomics study of 620 whole-exome sequencing: focusing on aspirin application]. Zhonghua Er Ke Za Zhi. 2016; 54(5):332-6. doi: 10.3760/cma.j.issn.0578-1310.2016.05.005.
25. Baclig MO, Predicala RZ, Mapua CA, et al. Allelic and genotype frequencies of catechol-O-methyltransferase (Val158Met) and CYP2D6*10 (Pro34Ser) single nucleotide polymorphisms in the Philippines. Int J Mol Epidemiol Genet. 2012; 3(2):115-21.
26. London SJ, Daly AK, Leathart JB, Navidi WC, Carpenter CC, Idle JR. Genetic polymorphism of CYP2D6 and lung cancer risk in African-Americans and Caucasians in Los Angeles County. Carcinogenesis. 1997; 18(6):1203-14.
27. Gao Y, Zhang Q. Polymorphisms of the GSTM1 and CYP2D6 genes associated with susceptibility to lung cancer in Chinese. Mutat Res. 1999; 444(2):441-9. Erratum in: Mutat Res. 2000; 464(2):311.
28. Perera FP, Tang D, Brandt-Rauf P, et al. Lack of associations between cancer and albumin adducts, ras p21 oncoprotein levels, and CYP1A1, CYP2D6, NAT1, NAT2 in a nested case-control study of lung cancer

within the physicians' health study. Cancer Epidemiol Biomarkers Prev. 2006; 15(7):1417-9.
29. He X, Feng S. Role of metabolic enzymes P450 (CYP) on activating procarcinogen and their polymorphisms on the risk of cancers. Curr Drug Metab. 2015; 16(10):850-63.
30. Agúndez JA, Gallardo L, Ledesma MC, et al. Functionally active duplications of the CYP2D6 gene are more prevalent among larynx and lung cancer patients. Oncology. 2001; 61(1):59-63.
31. Løvlie R, Daly AK, Molven A, Idle JR, Steen VM. Ultrarapid metabolizers of debrisoquine: characterization and PCR-based detection of alleles with duplication of the CYP2D6 gene. FEBS Lett. 1996; 392(1):30-4.
32. Banda Y, Kvale MN, Hoffmann TJ, et al. Characterizing race/ethnicity and genetic ancestry for 100,000 subjects in the Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort. Genetics. 2015; 200(4):1285-95. doi: 10.1534/genetics.115.178616.