

Original Article

Allelic and genotype frequencies of catechol-O-methyltransferase (Val158Met) and CYP2D6*10 (Pro34Ser) single nucleotide polymorphisms in the Philippines

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Abstract: A hospital-based cross-sectional study was conducted to determine the allelic and genotype frequencies in the genes encoding for catechol-O-methyltransferase and CYP2D6*10 among healthy volunteers and patients clinically diagnosed with cancer pain. PCR-RFLP was used to identify COMT and CYP2D6*10 genotypes. Allelic frequencies among healthy volunteer Filipinos were 0.83 and 0.17 for the COMT Val and COMT Met alleles, respectively. Calculated frequencies in Hardy-Weinberg equilibrium (HWE) were 73% for COMT Val/Val, 26% for COMT Val/Met, and 1% for COMT Met/Met genotype. For CYP2D6*10, allelic frequencies in HWE among volunteers were 0.46 for the C allele and 0.54 for the T allele. Twenty percent were identified as homozygous for the wild-type C/C genotype, 56% were identified as heterozygous for the C/T genotype, and 24% were identified as homozygous for the T/T variant genotype. No significant differences in COMT and CYP2D6*10 allele frequencies between cancer patients and healthy volunteers were noted. Our data demonstrated that the allele frequencies of COMT and CYP2D6*10 in the Filipino healthy volunteers were similar with other Asians but markedly different from Caucasian populations.

Keywords: Allele frequency, catechol-O-methyltransferase, CYP2D6*10, polymorphism, Filipino

Introduction

The human catechol-O-methyltransferase (COMT) gene is located in chromosome 22, band q11.2. Polymorphism in the gene encoding for COMT is linked to individual differences in pain sensitivity and response to opioid analgesics as well [1-3]. Depending on the combinations of the COMT alleles present, COMT genotypes may be classified as homozygote (Val/Val; Met/Met), or heterozygote (Val/Met) genotype. It has been shown that Caucasian cancer patients with Val/Val genotype require more morphine, while those with Met/Met require less opioids in order to effect relief from cancer pain. A guanine to adenine substitution at codon 158 in exon 3 of COMT causes a Valine to Methionine change leading to low Met/Met activity [4].

Cytochrome P450 2D6 (CYP2D6) located at 22q13.1, is one of the major drug metabolizing

genes involved in the biotransformation of many clinically important medications such as opioid analgesics. Phenotypic expressions include individuals with ultra-rapid, extensive, intermediate, and poor metabolizer status. Studies have shown that the various phenotypes have major effects on the efficacy of drugs as well as its adverse reactions. CYP2D6 genetic variation varies considerably within a population. Particularly, CYP2D6*10 allele is more common among Asians than among Caucasians. Four allelic variants of CYP2D6*10 are known such as *10A (CYP2D6J), *10B (CYP2D6Ch1), *10C (CYP2D6*36) and *10D (CYP2D6*37) which are collectively genotyped as CYP2D6*10 for diagnostic purposes [5-7]. A study among Chinese individuals showed that the allele frequency of CYP2D6*10 was about 52% to 55%. The C100T transition in exon 1 causes a Proline to Serine substitution leading to the formation of an unstable enzyme with lower activity [8, 9].

Table 1. Oligonucleotide primers used in COMT and CYP2D6*10 PCR amplification

SNP	Polarity	Nucleotide sequence (5' to 3')	Reference
COMT rs4680	Forward	ACT GTG GCT ACT CAG CTG TG	[10]
	Reverse	CCT TTT TCC AGG TCT GAC AA	
CYP2D6*10 rs1065852	Forward	GTG CTG AGA GTG TCC TGC C	[11]
	Reverse	CAC CCA CCA TCC ATG TTT GC	

Here we determined the allelic and genotype frequencies of COMT (Val158Met) and CYP2D6*10 (Pro34Ser) polymorphisms among Filipinos and compared the frequencies with different populations. In addition, we compared allelic and genotype frequencies of Filipino cancer patients with healthy volunteers to explore possible differences in the characteristics of polymorphisms between the two groups. To our knowledge, this is the first report of the allelic frequencies of COMT and CYP2D6*10 among Filipinos. This present study may provide useful information on the distribution of the two clinically important SNPs and also the data generated could be used to determine clinical outcomes in future studies.

Materials and methods

The study was approved by the Institutional Ethics Review Committee of St. Luke's Medical Center-Quezon City. All study participants gave informed consent prior to enrollment upon referral to the Pain Management Center. A total of 164 blood samples were genotyped for COMT and CYP2D6*10. Genomic DNA samples were obtained from 69 patients clinically diagnosed with cancer pathology stages 1 to 4, majority of which were breast and lung cancer, and 95 unrelated healthy volunteers from July 2008 to December 2011. Cancer pain patients were of interest as there are reports on differences in response to opioid analgesics depending on the genotype. There were 65 (40%) males and 99 (60%) female study participants with ages ranging from 21 to 83 years old. All cancer patients and healthy volunteers recruited for this study were Filipinos, ethnically and primarily of Malay (77%), Malay-Chinese (17%), and those 4th generation Filipino-European or Filipino-American descent (6%). Patients with Karnofsky performance scale of $\leq 30\%$, or ECOG 4 were excluded.

Blood was collected in EDTA-containing tubes

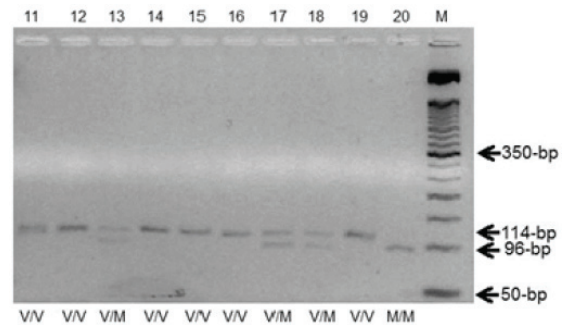


Figure 1. Gel Red-stained 2% agarose gel showing the digested PCR products used for the detection of COMT Val/Val (114-bp), Val/Met (114-bp and 96-bp) and Met/Met (96-bp) genotypes. Samples in lanes 11, 12, 14, 15, 16, and 19 are homozygous for the Val/Val genotype. Samples in lanes 13, 17, and 18 are heterozygous for the Val/Met genotype. Sample in lane 20 is homozygous for the Met/Met genotype. Molecular weight marker used is a 50-bp (Invitrogen) ladder.

and genomic DNA was extracted using the QIAamp DNA Kit. The primer sequence used in COMT amplification is shown in **Table 1** [10, 11]. PCR mixture consists of the following components: 10X PCR buffer, 10 mM dNTP, HotStarTaq DNA polymerase, 25 mM primers, and template DNA was made up to a volume of 50 μ l. Amplification of the COMT gene was carried out under the following conditions: initial denaturation at 95°C for 15 minutes, followed by 30 cycles at 94°C for 1 minute, 62°C for 1 minute, and 72°C for 1 minute. PCR products were digested with *Nla*III for 3 hours at 37°C. Amplicons were separated on 2% agarose gel stained with Gel Red (**Figure 1**).

The CYP2D6*10 gene amplification was carried with 20 mM each of the primers, 10X PCR buffer, 10 mM dNTP, HotStarTaq DNA polymerase, and DNA template. PCR was carried out at 95°C for 15 minutes, 56°C for 2 minutes,

72°C for 20 seconds, followed by 30 cycles at 94°C for 30 seconds, 56°C for 20 seconds, and 72°C for 20 seconds. The 325-bp amplicon was digested with *HphI* for 3 hours at 37°C (Figure 2).

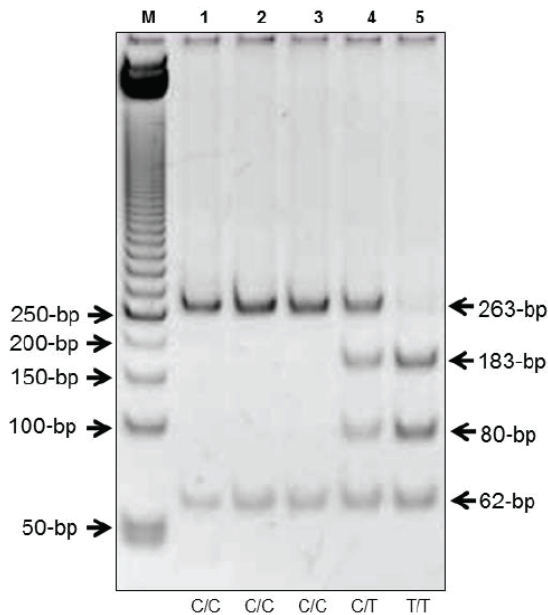


Figure 2. Ethidium bromide-stained 10% polyacrylamide gel showing digested amplicons used for CYP2D6*10 genotyping. Samples in lanes 1, 2, and 3 are homozygous (263-bp and 62-bp) for the wild-type C/C genotype. Sample in lane 4 is heterozygous (263-bp, 183-bp, 80-bp, 62-bp) for the C/T genotype, while sample in lane 5 is homozygous (183-bp, 80-bp, 62-bp) for the T/T variant genotype.

The amplicons of the COMT and CYP2D6*10 genes were purified using the MinElute PCR Purification Kit and sequenced using Big Dye Kit (1st BASE, Malaysia). Sequence data were aligned with the reference sequences from NCBI using BioEdit and ClustalW2 multiple sequence alignment (<http://www.ebi.ac.uk/Tools/msa/clustalw2>). GenBank accession numbers of COMT and CYP2D6*10 gene sequences used in the analysis were NG011526, AC000080, NM001025161, NM000106, and M33388. Nucleotide sequences reported in this study have been deposited to NCBI. It can be retrieved under GenBank accession numbers HQ262572 to HQ262574 and JQ801379 to JQ801380.

Data were analyzed using OpenEpi 2.2. The χ^2 test was used to determine significant differences in the allelic frequencies. Hardy-Weinberg equilibrium was tested using Fisher Exact Test χ^2 analysis for a 2x2 table. A p-value value below 0.05 was considered statistically significant throughout the population comparisons.

Results

Out of 167 samples collected, only 164 (98%) were genotyped. Three samples were not genotyped because of failure in PCR-RFLP analysis. **Table 2** shows the distribution of COMT and CYP2D6*10 genotypes among the study participants. Most individuals were found to be Val/Val (73%). Only 26% were genotyped as Val/Met, and 1% were Met/Met. For CYP2D6*10, 56% were found to be heterozygous for the C/T genotype.

Table 2. Distribution of study participants by COMT and CYP2D6*10 genotypes

Genotype	Cancer patients N (%)	Healthy volunteers N (%)	Total N (%)	p-value
COMT (Val158Met)				NS
Val/Val	56 (82)	64 (67)	120 (73)	
Val/Met	12 (17)	30 (32)	42 (26)	
Met/Met	1 (1)	1 (1)	2 (1)	
Total	69 (100)	95 (100)	164 (100)	
CYP2D6*10 (Pro34Ser)				NS
Homozygous wild-type C/C	15 (22)	18 (19)	33 (20)	
Heterozygous C/T	41 (59)	51 (54)	92 (56)	
Homozygous T/T variant	13 (19)	26 (27)	39 (24)	
Total	69 (100)	95 (100)	164 (100)	

NS = not significant

Table 3. Distribution of COMT and CYP2D6*10 genotypes in cancer patients and healthy volunteers

	COMT						CYP2D6*10					
	p	q	Val/Val (%)	Val/Met (%)	Met/Met (%)	Total	p	q	C/C (%)	C/T (%)	T/T (%)	Total
Cancer patients	0.90	0.10	56 (81)	12 (17)	1 (2)	69	0.51	0.49	15 (22)	41 (59)	13 (19)	69
Healthy volunteers	0.83	0.17	64 (67)	30 (32)	1 (1)	95	0.46	0.54	18 (19)	51 (54)	26 (27)	95
Total	0.86	0.14	120 (73)	42 (26)	2 (1)	164	0.48	0.52	33 (20)	92 (56)	39 (24)	164

p = frequency of Val
q = frequency of Met

p = frequency of allele C
q = frequency of allele T

COMT and CYP2D6*10 genotype frequencies of the cancer patients and healthy volunteers are shown in **Table 3**. Both COMT and CYP2D6*10 genotype frequencies were consistent with HWE in the two groups. No significant differences in COMT and CYP2D6*10 allele frequencies between cancer patients and healthy volunteers were noted. The allelic frequencies among healthy volunteer Filipinos were 0.83 and 0.17 for the COMT Val and COMT Met alleles, respectively. Among cancer patients, the allelic frequencies were 0.90 for the COMT Val and 0.10 for the COMT Met allele. The calculated frequencies in HWE were 73% for COMT Val/Val, 26% for COMT Val/Met and 1% for COMT Met/Met genotype. For CYP2D6*10, the allelic frequencies in HWE among healthy volunteers were 0.46 for the C allele and 0.54 for the T allele. Among cancer patients, the CYP2D6*10 allelic frequencies were 0.51 and 0.49 for the C and T alleles, respectively. Thirty three or (20%) were identified as homozygous for the wild-type C/C genotype, 92 (56%) were identified as heterozygous for the C/T genotype, and 39 (24%) were identified as homozygous for the T/T variant genotype.

Table 4 shows the COMT allele and genotype frequencies among various populations and the chi-square p-values resulting from the comparison of allele frequencies between the Filipinos and other ethnic populations. The result among Filipino healthy volunteers was used for the purpose of comparison with other populations. A significant difference was found in the COMT allele frequency between Filipinos and Cauca-

sians but not with other Asian populations.

In this study, the CYP2D6*10 allele frequencies among healthy volunteers were 0.46 for the C allele and 0.54 for the T allele. No significant differences were found between Filipinos and other Asians, except for South Indian, Chinese Taiwanese, and Japanese populations. Allelic frequencies of CYP2D6*10 were significantly different between Filipino and Caucasian populations as shown in **Table 5**.

Discussion

To our knowledge, the present study is the first to have documented the distribution of COMT and CYP2D6*10 alleles and genotypes among Filipinos. Previous studies have shown differences in CYP2D6 enzyme activity not only between major races but also among various Asian populations. Particularly, the CYP2D6*10 allele has been reported at frequencies of 52% to 55% among Chinese populations and 40% among Japanese populations [8, 9, 21]. Other studies reported that the CYP2D6*10 allele frequency among Singaporean and Chinese populations ranged from 62% to 70% [19, 20, 22]. Our data showed that allele frequency of CYP2D6*10 among Filipino healthy study participants were 54% and that majority of all the study participants were heterozygous for the C/T genotype. Differences in the allelic frequencies may reflect ethnic variety among Asian populations. Our findings also provided evidence that the allele frequencies for CYP2D6*10 among Filipinos were statistically

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Table 4. Allele and genotype frequencies of COMT reported from various ethnic populations.

Population	Method	Allele			Genotype Frequency (%)			Reference
		N	Frequency	p-value	Val/Val	Val/Met	Met/Met	
Asian								
Filipino	PCR-RFLP	95	0.17		67	32	1	Present study
Chinese	Sequencing	110	0.21	NS	60	37	3	[12]
Japanese	PCR	314	0.26	NS	54	39	7	[13]
Korean	PCR-RFLP	182	0.26	NS	53	41	6	[14]
Caucasian								
Norwegian	SSP-PCR	207	0.56	<0.0001	21	47	32	[2]
American	Taqman	202	0.50	<0.0001	25	51	24	[3]
French	Taqman	45	0.49	<0.0001	25	53	22	[15]
American	PCR-RFLP	1679	0.51	<0.0001	24	51	25	[16]
German	Taqman	50	0.50	<0.0001	24	52	24	[17]
Swedish	Taqman	43	0.47	0.0002	30	47	23	[18]

NS = not significant

Table 5. Allele frequencies of CYP2D6*10 reported from various ethnic populations.

Population	Method	N	Frequency	p-value	Reference
Asian					
Filipino	PCR-RFLP	95	0.54		Present study
Mainland Chinese	ASA	223	0.51	NS	[5]
Chinese	PCR-RFLP	70	0.52	NS	[8]
Chinese	ASA	295	0.55	NS	[9]
South Indian	PCR-RFLP	447	0.10	<0.0001	[11]
Chinese Taiwanese	PCR-RFLP	124	0.70	0.0123	[19]
Chinese Singaporean	PCR-RFLP	93	0.62	NS	[20]
Japanese	PCR	98	0.40	0.0531	[21]
Hong Kong Chinese	PCR-RFLP	119	0.64	NS	[22]
Korean	Sequencing	758	0.45	NS	[23]
Malaysian	PCR	107	0.49	NS	[24]
Thai	Multiplex PCR	288	0.44	NS	[25]
African					
African-American	PCR-RFLP	154	0.075	<0.0001	[26]
Ethiopian	PCR-RFLP	122	0.086	<0.0001	[27]
Caucasian					
American	PCR-RFLP	208	0.009	<0.0001	[28]
European	PCR-SSCP	672	0.014	<0.0001	[29]
Mexican-American	PCR-RFLP	349	0.074	<0.0001	[30]

NS = not significant

different from Caucasians. It has been shown that Asians have a high prevalence of the CYP2D6*10 intermediate allele ranging from 50% to 70% as compared with Caucasians [5, 31]. Our result is consistent with this reported high prevalence. Polymorphism in CYP2D6*10 is clinically important because of the greater likelihood of serious side effects or therapeutic failure due to poor metabolism of prodrug to the

active metabolite among individuals homozygous to the variant genotype [32-34]. Based on these previous results of this study, it is also likely that Filipinos who are heterozygous to the C/T genotype may experience lesser side effects or therapeutic failure as compared with Caucasian populations. However, the present study did not look into side effects and cannot confirm similar findings.

The COMT allele frequencies among Filipinos (0.17) were not significantly different from other Asian populations. It has been reported that the COMT allele frequencies in Chinese, Japanese, and Korean subjects were 0.21, 0.26, and 0.26, respectively [12-14]. On the other hand, our data showed that the allele frequency of COMT were markedly different from Caucasian populations. Diatchenko et al., and Schmahl et al., showed that the COMT allelic frequencies among American and German populations were 0.50 [3, 17].

Several methods for COMT and CYP2D6 genotyping have been used to identify SNPs including allele-specific amplification, multiplex PCR, DNA hybridization, DHPLC, and DNA chip-based assay [5, 7, 25, 33, 35]. In the present study, PCR-RFLP was used to identify COMT and CYP2D6*10 genotypes. PCR-RFLP despite being time-consuming, provides a useful and reliable method for identification of COMT and CYP2D6*10 genotypes. The guanine to adenine substitution at codon 158 of COMT as well as the CYP2D6*10 transition was confirmed by DNA sequencing.

In conclusion, no significant differences in COMT and CYP2D6*10 allele frequencies between cancer patients and healthy volunteers were noted. Our data demonstrated that the allele frequencies of COMT were similar with other Asian populations but markedly different from Caucasians. Allelic frequencies of CYP2D6*10 were statistically different from Caucasians but not with other Asian populations. From a clinical viewpoint, our findings may contribute to a better understanding of ethnic differences in opioid drug response, which may be used later on to predict and tailor individualized treatment among Filipinos.

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