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The Impact of Pharmacogenomics on Postoperative Nausea and Vomiting

Do CYP2D6 Allele Copy Number and Polymorphisms Affect the Success or Failure of Ondansetron Prophylaxis?

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Background: **Some patients treated with ondansetron for postoperative nausea and vomiting do not respond to therapy. One possible mechanism for this failure is ultrarapid drug metabolism via the cytochrome P-450 system, specifically the enzyme 2D6 (CYP2D6). Ultrarapid metabolism is seen in patients with multiple functional copies (≥ 3) of the CYP2D6 allele. This study was designed to determine whether patients who were given prophylactic ondansetron and had multiple CYP2D6 alleles had an increased rate of postoperative nausea and vomiting.**

Methods: **Two hundred fifty female patients undergoing standardized general anesthesia were given 4 mg ondansetron 30 min before extubation. Patients were observed for symptoms of nausea and vomiting. DNA was extracted from blood in all patients and was analyzed by using a gene-specific probe to determine the CYP2D6 gene copy number and genotyped by polymerase chain reaction amplification with a custom oligonucleotide microarray to determine the specific CYP2D6 genotypes.**

Results: **Eighty-eight patients experienced nausea, and 37 of those patients also had vomiting. In patients with one, two, or three CYP2D6 copies, the incidences of vomiting were 3 in 33 (27%), 27 in 198 (14%), and 7 in 23 (30%), respectively. The incidence of vomiting in subjects with three CYP2D6 copies was significantly different from those with two copies, but not from those with one copy. When analyzed by genotype, the incidences of vomiting in poor, intermediate, extensive, and ultrarapid metabolizers were 1 in 12 (8%), 5 in 30 (17%), 26 in 176 (15%), and 5 in 11 (45%), respectively ($P < 0.01$ *vs.* all other groups). There were no differences between groups in the incidence of nausea based on CYP2D6 copy number or genotype.**

Conclusions: **Patients with three copies of the CYP2D6 gene, a genotype consistent with ultrarapid metabolism, or both have an increased incidence of ondansetron failure for the prevention of postoperative vomiting but not nausea.**

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POSTOPERATIVE nausea and vomiting (PONV) is a frequent and unpleasant experience for patients undergoing surgery during general anesthesia. Approximately 20–40% of surgical patients experience PONV,[1] with certain high-risk groups having a PONV incidence as high as 80%.[2] The introduction of 5-hydroxytryptamine (5-HT_3) receptor antagonists for the treatment of PONV and chemotherapy-induced nausea and vomiting (CINV) has revolutionized the care of surgery patients and chemotherapy patients. However, although the 5-HT_3 antagonists have significantly reduced PONV, they have not totally eliminated it. For example, in several studies, high-risk patients were given a single 5-HT_3 antagonist, ondansetron, for prophylaxis of PONV, but patients still had a PONV rate of greater than 35%.[3,4] There are several mechanisms that can readily explain 5-HT_3 treatment failures, the most likely being the fact that PONV is multifactorial involving factors other than serotonin.[5] This is illustrated by the fact that multimodal PONV therapies directed against multiple targets have significantly increased efficacy over monotherapies.[6] This stands in contrast to CINV, which is mainly brought about by the release of serotonin and substance P.[7] Another cause for interindividual variations in drug response in the treatment of PONV may be variations in drug biotransformation by genetically polymorphic enzymes, such as the hepatic cytochrome P-450 enzyme 2D6 (CYP2D6).[8] All of the currently used 5-HT_3 antagonists are metabolized *via* cytochrome P-450 enzymes. It seems that the P-450 system has limited endogenous substrates and their primary function is the metabolism of dietary components, including drugs.[9] CYP2D6 is responsible for the majority of the metabolism of dolasetron and tropisetron[10] and partially responsible for the metabolism of ondansetron, which is also broken down by the enzymes CYP3A4, CYP2E1, and CYP1A2.[11] In contrast, granisetron, another 5-HT_3 antagonist, is primarily metabolized by CYP3A4, with no contribution from CYP2D6.[12]

CYP2D6 has a large number of reported polymorphisms and alleles that result in various phenotypic expressions of increased, decreased, or absent enzymatic activity.[13] Based on phenotypic behavior, the wild-type alleles are considered CYP2D6*1, CYP2D6*2, and CYP2D6*35. CYP2D6 activity may be classified into one of four categories: poor metabolizers (no enzyme production, with two deficient alleles), intermediate me-

tabolizers (possessing less activity than one wild-type allele), extensive metabolizers (two functional wild-type alleles) and ultrarapid metabolizers (possessing three or more functional wild-type alleles).[14] Chemotherapy patients who are ultrarapid metabolizers have been shown to have an increase in therapeutic failures with various agents, including the drugs tropisetron and ondansetron.[8] In general, polymorphisms in drug metabolizing enzymes may account for 10- to 10,000-fold variations in drug activity.[15] The exact phenotypic expression of a patient can be difficult to predict, especially in intermediate metabolizers, because of the large number of possible allele combinations and variable enzyme activity, which is often substrate specific. The frequency of each type of phenotypic metabolism subtype and polymorphic combinations is highly variable between populations of different geographic origins.[16]

Ondansetron, a 5-HT_3 antagonist that is commonly used to treat PONV, is partially degraded by CYP2D6. In patients undergoing chemotherapy, the drug's efficacy is reduced by an increase in the number of wild-type copies of the CYP2D6 gene.[8] We therefore hypothesized that patients who do not respond to ondansetron for prophylaxis of PONV may likewise possess an increased wild-type CYP2D6 gene copy number and are therefore ultrarapid metabolizers who degrade ondansetron more rapidly than those with two or fewer normally functioning alleles.

Materials and Methods

After obtaining University of Miami Institutional Review Board (Miami, Florida) approval and signed informed consent, 250 adult women aged between 18 and 64 yr with an American Society of Anesthesiologists physical status of I or II who were scheduled to undergo nonemergency surgery requiring general anesthesia of at least 30 min duration were enrolled. Patients provided medical histories and demographic information, including height, weight, age, ethnicity, tobacco use, secondhand smoke exposure, alcohol use, and menstruation history. Exclusion criteria included patients with known hypersensitivity to 5-HT_3 agents, a body mass index of 35 kg/m^2 or greater, significant systemic disease, nausea or vomiting within 24 h before the study, and use of antiemetics, steroids, H_2 antagonists, anticholinergics, antihistamines, butyrophenones, phenothiazines, or metoclopramide within 24 h of surgery.

Patients were premedicated with 1–2 mg midazolam in the holding area. Thiopental, 3–5 mg/kg, was used for induction, and 0.5–1 mg/kg succinylcholine, 0.5–1.2 mg/kg rocuronium, or 0.07–0.1 mg/kg vecuronium was used to facilitate neuromuscular blockade, with doses repeated as needed. All patients were intubated. Maintenance of anesthesia was achieved with less than 2.5% isoflurane, 50–70% nitrous oxide, and 2–10 μg/kg fentanyl. Oxygen concentrations were kept between 30 and 50% except for induction and just before extubation. Reversal of the neuromuscular blockade was achieved with 0.05 mg/kg neostigmine and 0.01 mg/kg glycopyrrolate, and every patient received reversal. All patients were given a prophylactic, open-label dose of 4 mg intravenous ondansetron approximately 30 min before extubation. Standard vital signs were monitored, and the use of gastric suction was noted. All patients were transported to the postanesthesia care unit with supplemental oxygen. Intravenous morphine, 1–4 mg (doses repeated as needed), was used for postoperative pain management in all patients for the first 4 h of the postoperative period. Patients received pain medication on request. Linear analog pain scores (0–10), 10 being the worst, were tracked for all patients at the time of arrival to the recovery room. If a patient experienced PONV, pain scores were recorded after treatment and at 6, 12, 18, and 24 h after extubation.

All patients were kept in a recovery room setting for 4 h. Each patient was questioned on arrival in the postanesthesia care unit if they were nauseated or felt like vomiting. Episodes of retching were considered equivalent to vomiting. Prophylactic treatment was considered a failure in any patient who reported feeling nauseated or had an episode of vomiting in the postanesthesia care unit. Patients who vomited or reported nausea and requested medication were treated and followed up for resolution of symptoms. Repeat episodes of vomiting or retching at intervals of greater than 1 min were considered separate events. The degree of nausea was graded on a linear analog scale of 0–10, with 10 being the worst. Patients whose symptoms did not improve or resolve within 30 min after administration of a rescue drug were given additional medication until symptoms were eliminated or the patient no longer requested treatment. All patients who required rescue medication were followed up for 24 h to determine whether the symptoms had resolved. If a patient did not experience nausea or vomiting within 4 h after extubation, the patient's participation in this study was ended, and prophylactic treatment was considered successful.

Determination of CYP2D6 Gene Copy Number

All patients participating in the study provided a 5-ml sample of blood, which was anticoagulated with EDTA and from which DNA was extracted using a QIAamp® DNA Mini Kit (Qiagen, Valencia, CA). Reagent components of the Invader® CYP450 Analysis System (Third Wave Technologies, Madison, WI) were used for analysis of CYP2D6 gene copy number. The method is based on the use of an Invader oligo and downstream gene-specific probe that hybridize in tandem to a CYP2D6 gene region where they create a structure that is a substrate for the Cleavase enzyme.[17] The target-specific cleaved

product from this primary reaction serves as an Invader oligo for a second reaction that generates a fluorescence resonance energy transfer (FRET) signal. CYP2D6-specific FRET signals were normalized to α-actin, a control reference gene not known to be duplicated or deleted. The relative fluorescent signals were used to determine CYP2D6 gene copy number. This Invader copy number assay does not distinguish between CYP2D6 polymorphic variants (CYP2D6*1, CYP2D6*2, CYP2D6*3, and so forth). Rather, it targets a conserved region of the CYP2D6 gene shared by all polymorphic variants and is designed to determine the number of copies of the CYP2D6 gene present in the patient's genome. The only known CYP2D6 allelic variant not detected by this assay is the CYP2D6*5 gene deletion allele. The numbers of CYP2D6 copies in this study included one copy (1×), two copies (2×), and three copies (3×). FRET signals from patient samples were grouped by clusters and compared with 1×, 2×, and 3× control calibrator genomic DNA samples provided by the manufacturer. Technicians and pathology staff were blinded to patient outcomes and histories; they possessed only sample numbers.

Genotyping CYP2D6 by Microarray Analysis

To determine which specific polymorphisms and alleles each patient possessed, a sample of DNA was analyzed using the AmpliChip CYP450 Assay (Roche Molecular Systems, Inc., Pleasanton, CA). The AmpliChip CYP450 assay combines a long multiplex polymerase chain reaction amplification with a custom oligonucleotide microarray manufactured by Affymetrix (Santa Clara, CA). After fragmentation and labeling of the polymerase chain reaction products, they are hybridized to the array. Hybridized amplicons are stained using streptavidin–phycoerythrin conjugate, and fluorescence associated with specific probe features is detected using a laser-illuminated, confocal scanner. Analysis of the pattern of hybridization to a series of probes that are specifically complementary to either wild-type or mutant sequences for each polymorphic site allows genotyping for 29 known mutations in the CYP2D6 gene. The assay is also capable of determining the presence of gene duplications and gene deletions. Specifically, the assay has been validated for detection of the presence of seven different known CYP2D6 duplication alleles, including *1,*2, *4, *10, *17, *35, and *41; however, the number of tandem copies (n) for the duplication alleles is not determined.[18] Technicians and all pathology staff were blinded to patient outcomes and histories. FRET assays and AmpliChip assays were run in different laboratories, and neither group knew of the other's results.

Statistical Analysis

This study was planned as a pilot to describe the CYP2D6 genotypes of patients who experienced PONV *versus* patients who did not. Data are presented as mean ± SE for continuous data and absolute frequencies (n) and percentages for frequency data. For statistical analysis, chi-square tests were used to compare proportions of patients, and univariate logistic regression was used for continuous predictor variables. All tests were two sided at a significance level of $P < 0.05$. Patients with significant protocol violations were not included in our analysis. Statistics were performed as a *post hoc* analysis.

Results

A total of 250 patients, of which 90 experienced PONV, were enrolled. Seven patients were withdrawn because of anesthesia-related protocol violations that could have influenced outcomes; 2 of the 7 were in the PONV group. One patient received the wrong induction agent, 2 patients received ondansetron more than 1 h before extubation, and 3 patients did not receive nitrous oxide or opioids. Another patient was withdrawn because of protracted hypotension in the postanesthesia care unit. Of the 243 patients remaining, 88 required PONV rescue within 4 h after extubation, a breakthrough rate of 36.2%. The breakthrough rate of vomiting alone was 15.2% (n = 37). All patients who vomited had nausea. Patient characteristics, PONV risk factors, and surgical data are reviewed in table 1.

Pharmacogenetics

Of the 243 blood samples of patients remaining in the intent-to-treat group 11 (4 of which had nausea but no vomiting, 7 of which had no breakthrough symptoms) were unavailable for complete analysis by both the Invader and AmpliChip assays. The remaining 232 were analyzed for allele copy number by FRET analysis, and the results are shown in table 2. Patients who possessed three copies (3×) of the CYP2D6 gene were more likely to have had an emetic episode despite ondansetron prophylaxis ($P = 0.034$) when comparing 3× patients with 2× patients. None of the patients tested demonstrated more than three copies of the CYP2D6 gene using the Invader copy number assay. Patients in the 1× group had a notable rate of PONV, but it did not prove to be statistically significant ($P = 0.21$). No samples were considered indeterminate.

The AmpliChip CYP450 analysis was performed on the same patient samples as the Invader copy number FRET analysis. The AmpliChip assay was able to provide both confirmation of the gene dosage as indicated by the FRET assay and a more detailed analysis of the exact polymorphisms each patient possessed. Table 3 lists the gene frequency in the study population. All patients demonstrating a 1× signal on the FRET analysis were shown to carry the CYP2D6*5 gene deletion allele on the

Table 1. Background Factors and Those Related to Surgery and Anesthesia

Variable	Patients with PONV (n = 88)		Patients without PONV (n = 155)		*P* Value
Age	42.95 (± 1.14)		44.57 (± 0.84)		0.22
BMI	26.48 (± 0.43)		27.11 (± 0.33)		0.25
No smoking	76	86.4%	119	76.8%	0.07
No second-hand smoke	74	84.1%	131	84.5%	0.93
History of PONV	35	39.8%	24	15.4%	< 0.0001*
History of motion sickness	31	35.2%	39	25.2%	0.10
No alcohol use	76	86.4%	144	93%	0.10
NGT used	30	34.1%	43	27.7%	0.30
Time from ondansetron to extubation	00:35:57 (± 1:37 min)		00:39:30 (± 1:49 min)		0.18
Duration of surgery	2:21:10 (± 6.92 min)		2:43:35 (± 5.64 min)		0.08
Pain score on arrival in PACU	4.9 (± 0.42)		4.1 (± 0.31)		0.12
No. of menopausal patients	23	26.1%	46	29.7%	0.56
Days since last menstrual period	17.8 (± 0.43)		17.2 (± 0.86)		0.74
Morphine use 4 h (mg)	6.3 (± 0.55)		9.4 (± 0.64)		0.02†
Type of surgery					
Breast	15	17.0%	22	14.2%	
Abdominal	11	12.5%	12	7.7%	
Orthopedic	1	1.1%	1	< 1%	
Gynecologic	50	56.8%	95	61.3%	
Urologic	5	5.7%	6	3.9%	
Plastics	3	3.4%	7	4.5%	
ENT	3	3.4%	12	7.7%	

Values are presented as mean (± SE), number, and percentage.

* $P < 0.001$ for patients with PONV *vs.* patients without PONV. † $P = 0.02$ for patients with PONV *vs.* patients without PONV.

BMI = body mass index; ENT = ear, nose, and throat; NGT = nasogastric tube; PACU = postanesthesia care unit; PONV = postoperative nausea and vomiting.

AmpliChip assay (100% concordance). This finding is consistent with the design of the Invader copy number test, which does not recognize or bind to the CYP2D6*5 allele. The presence of CYP2D6*5 is therefore inferred from the 1× signal in a compound heterozygote (where one of the alleles is CYP2D6*5). The AmpliChip CYP450 assay further allowed us to clarify the status of those patients designated as 2× by indicating the presence of nonfunctioning and reduced activity alleles other than CYP2D6*5. Using the AmpliChip data, any patient possessing two nonfunctioning alleles (such as CYP2D6*4) was designated as a poor metabolizer, any patient possessing at least one decreased but functioning allele with no wild-type alleles was designated as an intermediate metabolizer, any patient with at least one wild-type allele (but less than three) was designated as an extensive metabolizer, and any patient with three or more normally functioning alleles (*i.e.*, combinations of *1, *2, *35) was designated as an ultrarapid metabolizer. When groups were compared (table 4) we found that the ultrarapid metabolizers clearly had an increased incidence of emesis over not only the extensive metabolizers ($P = 0.008$) but all groups combined ($P = 0.007$).

Table 2. CYP2D6 Gene Copy Number by FRET and the Incidence of PONV

CYP2D6 Copy Number	Allele Frequency	Vomiting	Nausea ± Vomiting
1X	11/232 (4.7)	3/11 (27.3)	5/11 (45.5)
2X	198/232 (85.3)	27/198 (13.6)	70/198 (35.4)
3X	23/232 (9.9)	7/23 (30.4)*	9/23 (39.1)

Values are presented as number (percentage).

* $P = 0.034$ for 3X *vs.* 2X for vomiting.

1X = one allele copy; 2X = two allele copies; 3X = three allele copies; FRET = fluorescence resonance energy transfer; PONV = postoperative nausea and vomiting.

Table 3. CYP2D6 Allele Frequency in Study Population

Allele	Occurrence	All Participants (n = 229)
*1	178	0.388
*2	69	0.151
*3	2	0.004
*4	60	0.131
*5	11	0.024
*6	2	0.004
*7	1	0.002
*9	7	0.015
*10	13	0.029
*14	1	0.002
*17	26	0.057
*29	15	0.033
*35	11	0.024
*36	1	0.002
*39	1	0.002
*40	2	0.004
*41	36	0.079
*1xn	5	0.011
*2xn	11	0.024
*4Dxn	4	0.009
*9xn	1†	0.002
*41xn	1	0.002

Values are presented as number and frequency.

† Sample needs genotype confirmation by an independent method.

xn = duplication of gene.

Table 4. Metabolizer States as Determined by Amplichip Analysis and Incidence of PONV

Metabolizer Status	Frequency	Vomiting	Nausea ± Vomiting
Poor metabolizers	12/229 (5.2)	1/12 (8.3)	5/12 (41.7)
Intermediate metabolizers	30/229 (13.1)	5/30 (16.7)	7/30 (23.3)
Extensive metabolizers	176/229 (76.9)	26/176 (14.7)	65/176 (36.9)
Ultrarapid metabolizers	11/229 (4.8)	5/11 (45.5)*	5/11 (45.5)
Indeterminate	3	—	—

Values are presented as number (percentage).

* $P = 0.007$ *vs.* all other groups for vomiting.

PONV = postoperative nausea and vomiting.

Once again, nausea did not prove to be related to genotype (ultrarapid metabolizers *vs.* extensive metabolizers; $P = 0.58$). Initial nausea scores for patients designated as poor, intermediate, extensive, and ultrarapid metabolizers were 1.8, 2.4, 3.2, and 1.6, respectively. No consistent differences in PONV risk factors were found between ultrarapid metabolizers who developed PONV and those who did not develop PONV.

There were only 2 samples of 232 that showed discordance between the Invader copy number analysis and the AmpliChip (99.1% concordance). One sample showed a 2× Invader FRET signal and was shown to be an ultrarapid metabolizer by the AmpliChip CYP450 analysis, whereas the other sample showed a 3× Invader FRET signal but by AmpliChip gave a genotype result consistent for 2X, specifically CYP2D6*10B/*10B. Overall, 3 of the 232 samples analyzed by the Invader copy number assay could not be analyzed by the AmpliChip CYP450 assay. These samples returned "No call" results for previously unknown polymorphism combinations. A significant finding was that only 10 (43.5%) of the 23 patients designated as 3× by the Invader copy number assay were found to carry ultrarapid metabolizer phenotypes. Of the 12 samples that were not found to be ultrarapid metabolizers, 5 proved to have genotypes that were consistent with extensive metabolizers, 5 more samples had genotypes consistent with intermediate metabolizers, and 2 samples demonstrated a genotype for poor metabolizers. One 3× sample on FRET was indeterminate by the AmpliChip assay. Although not yet confirmed by another method, the AmpliChip CYP450 analysis provided results consistent with the presence of a previously unreported duplication of the CYP2D6*9 allele. When the patients characterized as 3× by the Invader assay (but not phenotypic ultrarapid metabolizers by AmpliChip analysis) were removed, it was noted that all 5 (100%) of the ultrarapid metabolizers who experienced PONV also vomited (table 5).

Table 5. Genotypes and Outcomes of Patients Designated as 3X by FRET or UM by Amplichip

Patient Number	FRET Copy Number	Amplichip Genotype	Vomiting	Nausea ± Vomiting	Predicted Phenotype
1	2X	1xn/2A	Yes	Yes	UM
2	3X	1xn/2A	Yes	Yes	UM
3	3X	1/2Axn	Yes	Yes	UM
4	3X	1/2Axn	Yes	Yes	UM
5	3X	2A/2Axn	Yes	Yes	UM
6	3X	1/2Axn	No	No	UM
7	3X	2A/2Axn	No	No	UM
8	3X	1xn/2A	No	No	UM
9	3X	1/2Axn	No	No	UM
10	3X	1/2Axn	No	No	UM
11	3X	1/2Axn	No	No	UM
12	3X	2Axn/4A	No	No	EM
13	3X	1xn/41	No	No	EM
14	3X	1xn/29	No	Yes	EM
15	3X	10B/2Axn	No	No	EM
16	3X	29/2Axn	No	No	EM
17	3X	10B/10B	No	Yes	IM
18	3X	4A/9xn	No	No	IM
19	3X	41/41xn	No	No	IM
20	3X	41/4Dxn	Yes	Yes	IM
21	3X	17/4Dxn	Yes	Yes	IM
22	3X	4A/4Dxn	Yes	Yes	PM
23	3X	4A/4Dxn	No	No	PM
24	3X	Indeterminate	No	No	?

EM = extensive metabolizer; FRET = fluorescence resonance energy transfer; IM = intermediate metabolizer; PM = poor metabolizer; UM = ultrarapid metabolizer; xn = gene duplication.

Discussion

5-Hydroxytryptamine receptor antagonists have revolutionized PONV and CINV treatment. These agents have a high efficacy with a low incidence of adverse effects. Unfortunately, not every patient has a beneficial response when treated. This failure to respond seems in part to be due to interindividual genetic variations in the CYP2D6 gene or other as-yet-uncharacterized variations. Therefore, a patient's genetic makeup may hold the key to their successful medical therapy.

In this study, we demonstrated that patients who possess three functional copies of the CYP2D6 allele are more likely to experience vomiting but not necessarily nausea in the postoperative period despite the prophylactic administration of ondansetron. The fact that vomiting but not nausea increased significantly is not unexpected because ondansetron has previously been shown to be a better antiemetic than an antinausea agent.[19] Other studies evaluating the effects of CYP2D6 polymorphisms on therapeutic failures for 5-HT_3 agents in CINV reported similar results. For example, in one study, pa-

tients with three copies of the CYP2D6 gene who received tropisetron had a significantly higher mean number of vomiting episodes than all other patients in the early observational period. In addition, these authors observed that the effects for the CYP2D6 ultrametabolizers were similar for treatment with tropisetron as well as ondansetron.[8]

The mechanism for failure of prophylactic ondansetron therapy in a CYP2D6 ultrarapid metabolizer is presumed to be increased metabolism and clearance of the primary drug by this pathway. This stands in contrast to poor and extensive metabolizers, who seem to eliminate drug at a similar rate primarily *via* pathways other than CYP2D6.[20] Although ondansetron is not metabolized solely by CYP2D6, this enzyme probably plays a more significant role in rapid drug elimination in patients who are ultrarapid metabolizers. Because ondansetron concentrations were not determined at the time of PONV, no specific comments about the correlation between exact drug concentrations and breakthrough can be made. However, it has been previously reported that patients with CYP2D6 wild-type allele duplications have lower 5-HT_3 drug concentrations and a higher rate of CINV compared with patients with two or fewer active genes, and there is no reason to suspect that this is not the case with PONV as well.[8] Although other studies, performed on healthy subjects, support the theory of increased drug elimination in subjects with CYP2D6 duplications,[13] there is limited evidence demonstrating a correlation between serum ondansetron concentrations and effect. In addition, the antiemetic effect of ondansetron seems to outlast its 3- to 4-h half-life.[19] One possible explanation for this might be that serum concentrations may not reflect the concentration of the drug at its site of action.

Most of the previously established risk factors for PONV, although showing a trend in our study, were not statistically significant. As has been repeatedly shown by other investigators, our patients with a previous history of PONV were also clearly at the greatest risk of PONV.[21] Paradoxically, other risk factors for PONV, such as duration of surgery, seemed to have a higher trend in patients who did not have symptoms of PONV. In addition, morphine use seemed inversely related to the risk of PONV and was statistically significant.

Both the Invader gene copy number and AmpliChip CYP450 assays for CYP2D6 genetic analysis proved to be useful and had a high degree of concordance for the determination of CYP2D6 gene copy number. The Invader copy number analysis itself only gave the most basic information in terms of gene dosage and did not report the presence of any CYP2D6 polymorphisms other than the *5 gene deletion allele. Therefore, a simple gene dosage assay could be misleading if it were used to try to analyze an individual patient in the absence of more comprehensive genotype analysis and might better serve as a screening tool. A comprehensive CYP2D6 genotyping strategy using Invader assay coupled with polymerase chain reaction amplification has been developed and can address the need for more precise testing with the Invader assay.[17]

The AmpliChip CYP450 assay allowed us to genotype patients for the presence of 29 CYP2D6 polymorphisms and 33 alleles, including gene deletion and seven different gene duplication alleles. By looking at the specific polymorphisms, rather than just gene copy number, we could ascertain whether the gene copy number was clinically significant, predicting either a poor metabolizer or an ultrarapid metabolizer phenotype. This proved to be especially true in the case of discerning which gene duplication carriers were ultrarapid metabolizers. Although the Invader copy number assay and AmpliChip CYP450 assay varied greatly by technique, they clearly gave overall similar copy number results for our study, thus providing parallel independent confirmation of the validity of our results.

In conclusion, this study suggests that antiemetic treatment for postoperative vomiting may be made more efficacious by selecting a 5-HT_3 antagonist and/or dose or another class of antiemetic drug that is consistent with the patient's CYP2D6 genotype. Specifically, patients with three normally functioning CYP2D6 alleles might benefit from the use of antiemetics that are not metabolized by this enzyme. The ultimate determination of the usefulness of these pharmacogenetic studies depends on whether the information can be used economically and effectively to reduce patient complications and treatment failures.

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