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ORIGINAL ARTICLE

# Association of *ABCB1* polymorphisms with the efficacy of ondansetron for postoperative nausea and vomiting

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## Summary

We investigated whether the 2677G>T/A and 3435C>T polymorphisms of adenosine triphosphate-binding cassette subfamily B member 1 (*ABCB1*) affect the efficacy of ondansetron to prevent postoperative nausea and vomiting. One hundred and ninety-eight patients undergoing general anaesthesia were enrolled. Thirty minutes before the end of surgery, 0.1 $mg.kg^{-1}$ ondansetron was administered intravenously. The incidence of postoperative nausea and vomiting was compared between genotypes in the 2677G>T/A and 3435C>T polymorphisms of *ABCB1*. The incidence of postoperative nausea and vomiting was lower in patients with the 2677TT genotype (TT vs Non-TT = 25.9% vs 53.0%, p = 0.01) and 3435TT genotype (CC + CT vs TT = 52.6% vs 21.7%, p = 0.01) during the first 2 h after surgery. There were no significant differences in the incidence of postoperative nausea and vomiting between the different genotype groupings during period between 2 and 24 h after surgery. In conclusion, *ABCB1* genotypes may be a clinical predictor of responsiveness for ondansetron.

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Postoperative nausea and vomiting (PONV) is a common and distressing complication in patients undergoing general anaesthesia. The incidence ranges between 20% and 30%, but is as high as 80% in high-risk patients [1] and may cause significant complications [2]. Although the exact mechanism of PONV is not clear, it is known that the chemoreceptor trigger zone in the area postrema plays an important role, in which the 5-hydroxytryptamine type-3 (5-$HT_3$) receptor is involved in the occurrence of PONV [3].

Currently, 5-$HT_3$ receptor antagonists such as ondansetron are widely used for prevention and treatment of PONV and although these agents have a significant effect, over 35% of patients treated with ondansetron may still experience PONV [4, 5]. We hypothesised that polymorphism in the adenosine triphosphate-binding cassette subfamily B member 1 (*ABCB1*), an encoding gene of transporter for ondansetron in the brain-blood barrier [6], would contribute to such inter-individual variation.

In a previous study, the 3435C>T single nucleotide polymorphism of *ABCB1* was associated with the efficacy of 5-$HT_3$ receptor antagonists for chemotherapy-induced nausea and vomiting [7]. Therefore, we investigated whether the 2677G>T/A, and 3435C>T polymorphisms of *ABCB1* affect the efficacy of ondansetron in preventing PONV in patients undergoing general anaesthesia.

## Methods

This study was approved by the Institutional Review Board of Yonsei University Hospital. Written informed consent was obtained from each patient. One hundred and ninety-eight adult patients, of ASA physical status 1–2, undergoing laparoscopic cholecystectomy were enrolled in this study. Patients with a history of drug abuse, known hypersensitivity to 5-$HT_3$ receptor antagonist, body mass index > 30 $kg.m^{-2}$, hepatic or renal disease or who had used antiemetics within the 24 h before the study were excluded. Before surgery, all patients were interviewed about their medical history including tobacco use, presence of PONV and motion sickness.

No premedication was administered to the patients. Electrocardiography, noninvasive blood pressure and pulse oximetry were monitored continuously after the patient's arrival in the operating theatre. Anaesthesia was induced with thiopental 4–5 $mg.kg^{-1}$ and remifentanil 1.0 $\mu g.kg^{-1}$ intravenously, and rocuronium 0.6 $mg.kg^{-1}$ was given to facilitate tracheal intubation. The patient's lungs were ventilated with 50% oxygen in air. Sevoflurane 1.5–2.0 vol% and remifentanil 0.1–0.2 $\mu g.kg^{-1}.min^{-1}$ were used for maintenance of anaesthesia. A bispectral index score (BIS) monitor (A-2000 BIS Monitor™; Aspect Medical System Inc., Newton, MA, USA) was used continuously during the procedure and depth of anaesthesia maintained appropriately. A forced-air warming system (Bair-Hugger™; Augustine-Medical, Eden Prairie, MN, USA) was used to maintain body temperature at 36.0–37.0 °C. Thirty minutes before the end of surgery, ondansetron 0.1 $mg.kg^{-1}$ was administered intravenously, and ketorolac 60 mg was given. The total dose of remifentanil consumption during anaesthesia, and the pain score on arrival in the post-anaesthesia care unit, were recorded.

We noted any episode of nausea or vomiting during the first 2 h and between 2 and 24 h after surgery. Patients who vomited or suffered from a feeling of nausea were treated with rescue antiemetics. The primary endpoint of this study was the proportion of patients who did not experience any episode of nausea or vomiting in each group of genotypes.

Genomic DNA was prepared from peripheral blood samples using a nucleic acid isolation device, QuickGene-mini80 (FUJIFILM, Tokyo, Japan). The genotyping of 2677G>T/A and 3435C>T *ABCB1* gene variants was screened using single base primer extension assay using ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to the manufacturer's recommendation. Briefly, the genomic DNA was amplified with polymerase chain reaction (PCR) with forward (F) and reverse (R) primer pairs and standard PCR reagents in a 10-μl reaction volume, containing 10 ng of genomic DNA, 0.5 pM of each oligonucleotide primer, 1 μl of 10× PCR buffer, 250 μM dNTP and 0.25 U i-StarTaq DNA polymerase (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea). Primers were designed on the basis of target gene sequences: 2677G>T/A: 5′-CAG-GAAACAGCTATGACCTCAGCATTCTGAAGTCA-TGGA-3′(F), 5′-TGTAAAACGACGGCCAGTTCCA-AGAACTGGCTTTGCT-3′(R) and 3435C>T: 5′-TG-TTTGACTGCAGCATTGC-3′(F), 5′-TTTATTTGA-AGAGAGACTTACATTAG GC-3′(R). The PCR reactions were carried out as follows: one cycle at 95 °C for 10 min, and 35 cycles at 95 °C for 30 s, 55 °C for 1 min, 72 °C for 1 min followed by one cycle of 72 °C for 10 min. After amplification, the PCR products were treated with 1 U each of shrimp alkaline phosphatase (SAP) (Roche, Roche Applied Science, Mannheim, Germany) and exonuclease I (USB Corporation, Cleveland, OH, USA) at 37 °C for 75 min and 72 °C for 15 min to purify the amplified products. One microlitre of the purified amplification products was added to the SNaPshot Multiplex Ready reaction mixture containing 0.15 pM of genotyping primer (2677G>T/A: TATTTAGTTTGACTCACCTTCCCAG, 3435C>T: TGTTGGCCTCCTTTGCTGCCC TCAC) for primer extension reaction. The primer extension reaction was carried out for 25 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 30 s. The reaction products were treated with 1 U of SAP at 37 °C for 1 h and 72 °C for 15 min to remove excess fluorescent dye terminators. One microlitre of the final reaction samples containing the extension products was added to 9 μl of Hi-Di formamide (ABI). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analysed by electrophoresis in an ABI Prism 3730xl DNA analyser (Applied Biosystems, Foster City, CA, USA). Analysis was carried out using GENEMAPPER software (version 4.0; Applied Biosystems).

Considering the expected frequency of TT genotype to be 17% based on a previous study [8], we estimated that to achieve a power of 90% ($\alpha = 0.05$, $\beta = 0.1$), assuming a difference of 30% in the failure rate for ondansetron treatment among the genotypes, 180 patients would be required. Therefore, we recruited 198 patients to compensate for excluded patients. The frequencies of the two single nucleotide polymorphisms were assessed for deviation from Hardy–Weinberg equilibrium using Fisher's exact test. Frequency differences in genotype, demographic data, and incidence of PONV were compared by chi-squared test, Fisher's exact test, or ANOVA with Bonferroni correction as appropriate. Haplotypes were estimated using HAPLOVIEW 3.2 (Broad Institute, Cambridge, MA, USA) based on a standard expectation-maximisation algorithm. Data were analysed using SPSS version 12.5 (SPSS Inc., Chicago, IL, USA). A value for $p < 0.05$ was considered significant.

## Results

Patients' characteristics and clinical data according to *ABCB1* genotypes are summarised in Tables 1 and 2. There were no significant differences in characteristics and clinical data according to genotypes. Frequencies of the *ABCB1* 2677 genotypes were as follows: 21.3% for GG; 34.5% for GT; 16.8% for GA; 13.7% for TT; 10.2% for TA; and 3.6% for AA. Frequencies of the *ABCB1*

**Table 1** Patients' characteristics and clinical details according to *ABCB1* 2677 genotype. Values are mean (SD) or number.

| | Genotype | | |
|---|---|---|---|
| Variable | Non-T (*n* = 82) | TG or TA (*n* = 88) | TT (*n* = 27) |
| Sex; M/F | 37/45 | 31/57 | 13/14 |
| Age; years | 46.0 (9.3) | 48.3 (9.8) | 48.9 (9.6) |
| History of smoking | 12 | 9 | 4 |
| History of PONV | 1 | 1 | 0 |
| History of motion sickness | 27 | 24 | 9 |
| Duration of surgery; min | 72.9 (24.8) | 68.5 (28.4) | 76.5 (44.5) |
| Remifentanil doses in operating room; μg | 516.3 (206.9) | 528.9 (207.0) | 545.9 (337.1) |
| Pain score in post-anaesthesia care unit | 5.9 (1.8) | 6.2 (2.4) | 5.9 (2.2) |

**Table 2** Patients' characteristics and clinical details according to *ABCB1* 3435 genotype. Values are mean (SD) or number.

| | Genotype | | |
|---|---|---|---|
| Variable | CC (*n* = 81) | CT (*n* = 94) | TT (*n* = 23) |
| Sex; M/F | 37/44 | 31/63 | 13/10 |
| Age; years | 45.7 (8.4) | 48.5 (10.5) | 48.4 (9.8) |
| History of smoking | 13 | 8 | 4 |
| History of PONV | 1 | 2 | 0 |
| History of motion sickness | 26 | 26 | 9 |
| Duration of surgery; min | 74.2 (27.9) | 67.1 (24.4) | 79.1 (48.4) |
| Remifentanil doses in operating room; μg | 533.1 (235.6) | 506.3 (172.6) | 576.1 (361.5) |
| Pain score in post-anaesthesia care unit | 5.9 (1.9) | 6.2 (2.2) | 5.6 (2.1) |

3435 genotypes were as follows: 40.9% for CC; 47.5% for CT; and 11.6% for TT. The genotype frequencies of all single nucleotide polymorphisms were in Hardy–Weinberg equilibrium ($p > 0.05$). Frequencies of the haplotypes were as follows: 45.2% for 2677G-3435C; 32.5% for 2677T-3435T; 16.0% for 2677A-3435C; 3.6% for 2677T-3435C; 1.8% for 2677G-3435T; and 1.0% for 2677A-3435T.

Among 2677G>T/A variants, the incidence of PONV during the first 2 h after surgery was lower in patients with the 2677TT genotype than other 2677 genotypes (TT vs Non-TT, $p = 0.01$) (Table 3). For 3435C>T variants, it was lower in patients with the 3435TT genotype than other 3435 genotypes during the first 2 h after surgery (CC + CT vs TT, $p = 0.01$) (Table 4). In haplotype analysis, there were no significant differences in the incidence of PONV according to haplotype (Table 5). There were no significant differences in the incidence of PONV between the different genotype grouping during the period between 2 and 24 h after surgery.

**Table 3** Effects of *ABCB1* 2677 genotype on the efficacy of ondansetron for postoperative nausea and vomiting. Values are number (proportion).

| | First 2 h | | 2–24 h | |
|---|---|---|---|---|
| | Responder | Non-responder | Responder | Non-responder |
| Genotypes (*n* = 197) | | | | |
| GG | 18 (42.9%) | 24 (57.1%) | 37 (88.1%) | 5 (11.9%) |
| GT | 32 (47.1%) | 36 (52.9%) | 58 (85.3%) | 10 (14.7%) |
| GA | 20 (60.6%) | 13 (39.4%) | 31 (93.9%) | 2 (6.1%) |
| TT | 20 (74.1%) | 7 (25.9%) | 25 (92.6%) | 2 (7.4%) |
| TA | 8 (40%) | 12 (60%) | 17 (85%) | 3 (15%) |
| AA | 3 (42.9%) | 4 (57.1%) | 7 (100%) | 0 (0%) |
| p values | 0.03 | | 0.41 | |
| Non-TT vs TT | 0.01 | | 0.75 | |

**Table 4** Effects of ABCB1 3435 genotype on the efficacy of ondansetron for postoperative nausea and vomiting. Values are number (proportion).

| | First 2 h | | 2–24 h | |
|---|---|---|---|---|
| | Responder | Non-responder | Responder | Non-responder |
| Genotypes (*n* = 198) | | | | |
| CC | 38 (46.9%) | 43 (53.1%) | 72 (88.9%) | 9 (11.1%) |
| CT | 45 (47.9%) | 49 (52.1%) | 81 (86.2%) | 13 (13.8%) |
| TT | 18 (78.3%) | 5 (21.7%) | 22 (95.7%) | 1 (4.3%) |
| p values | | | | |
| CC + CT vs TT | 0.01 | | 0.32 | |
| CC vs CT + TT | 0.39 | | 1.00 | |

**Table 5** Effects of major 5 *ABCB1* haplotypes on the efficacy of ondansetron for postoperative nausea and vomiting. Values are proportion of total haplotype numbers. No significant differences.

| | First 2 h | | 2–24 h | |
|---|---|---|---|---|
| | Responder | Non-responder | Responder | Non-responder |
| Haplotype (2677–3435) | | | | |
| G-C | 21.1% | 24.1% | 39.6% | 5.6% |
| T-C | 1.8% | 1.8% | 2.8% | 0.8% |
| A-C | 7.9% | 8.1% | 14.7% | 1.3% |
| G-T | 1.3% | 0.5% | 1.8% | 0% |
| T-T | 17.5% | 15% | 28.9% | 3.6% |

## Discussion

Our results demonstrate that there was a significant association between *ABCB1* gene polymorphism and the response to ondansetron. The incidence of PONV was lower in patients with 2677TT and 3435TT genotypes than other genotypes during the first 2 h after surgery.

The *ABCB1* transporter, known as P-glycoprotein (P-gp) or multidrug resistance gene1 (*MDR1*), is a transmembrane protein that functions as an efflux pump in many tissues including the brain-blood barrier [6, 9, 10]. It is considered to be an important transporter that limits the accumulation of various drugs such as chemotherapeutic and antiepileptic drugs [11–13]. Some 5-$HT_3$ receptor antagonists including ondansetron are also substrates for P-gp [6, 14]. *ABCB1* polymorphisms may affect the expression and function of P-gp, and variable expression of P-gp can influence the drug disposition in the central nervous system, and thus efficacy. For example, *ABCB1* knockout mice show the absence of P-gp in the brain-blood barrier, which causes a higher brain accumulation of ivermectin, resulting in drug-induced neurotoxicity [15]. Siddiqui et al. [16] demonstrated that the TT genotype at *ABCB1* 3435 was associated with a better drug response in epilepsy patients. Therefore, it is reasonable to hypothesise that genetic variance of this transporter could influence the efficacy of ondansetron.

There is some evidence that genetic polymorphism may influence the antiemetic efficacy of ondansetron [7, 17–19]. In a recent study, *ABCB1* 3435C>T polymorphism was associated with the antiemetic efficacy of 5-$HT_3$ receptor antagonists in patients undergoing chemotherapy treatment [7]. We also found a significant association between 2677G>T/A and 3435C>T polymorphisms in *ABCB1* and the efficacy of ondansetron for PONV during first 2 h after surgery. It is likely that patients with 2677TT and/or 3435TT genotype have a higher concentration of ondansetron in the central nervous system and better response to ondansetron due to reduced activity of *ABCB1* transporter. If it is possible to predict the responsiveness of PONV to ondansetron, it may be possible to target treatment appropriately. However, further studies are required to evaluate the effect of genetic polymorphism in the *ABCB1* gene on P-gp expression in the blood-brain barrier.

We could not find any significant association between haplotypes and incidence of PONV. However, patients with 2677TT or 3435TT genotype were most responsive to ondansetron in our study. Therefore, we suggest that the TT genotype is the major determinant of the response to ondanstron prophylaxis for PONV.

We found that the response to ondansetron for PONV did not differ according to genotypes during between 2 h and 24 h after surgery. However, the incidence of PONV in that time period was < 10% of patients.

It is known that there is a strong linkage disequilibrium between the *ABCB1* 2677G>T/A, and 3435C>T alleles in Asians, especially in Koreans [8, 20]. In this study, we found that allele and genotype frequencies of two common variants of the *ABCB1* gene were similar to those in a previous study on Koreans.

There are several risk factors affecting PONV. It is well known that sex, a history of motion sickness or PONV, non-smoking and the use of opioids are the major risk factors for PONV [21]. In this study, there were no significant differences in these risk factors according to genotypes. Moreover, since other risk factors that affect PONV, such as anaesthetic agent and type of surgery, were also controlled in this study, they would have minimal effect on our results. For example, we maintained BIS values, indicating the degree of anaesthetic depth, between 50 and 60. Thus, any potential influences of anaesthetic agents or techniques as used in this study on the results were probably insignificant.

In conclusion, the response to ondansetron for PONV was significantly influenced by *ABCB1* gene 2677G>T/A, and 3435C>T polymorphisms. *ABCB1* genotypes may be a clinical predictor of responsiveness for ondansetron for PONV.

## Competing interests

No competing interests and no external funding declared.

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