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Pharmacogenetics of antiemetics in Indonesian cancer patients

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4

Differences in 5-Hydroxytryptamine-3B haplotype frequencies between Asians and Caucasians

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ABSTRACT

Background: The 5-hydroxytryptamine 3 (5-HT3) receptor is a ligand-operated ion channel with five different receptor subunits (5-HT3A, B, C, D, and E) found in humans. Activation of 5-HT3 receptors causes various effects such as drug-induced emesis and behavioral effects such as anxiety, depression and cognitive disorders. To explore interethnic differences in 5-HT3 receptor antagonists response, we studied haplotype frequencies in the gene encoding the 5-HT3B receptor in Asians and Caucasians.

Methods: Three SNPs of *5-HT3B* receptor gene, deletion AAG in 5'-UTR position, 18792A>G in intron position and 46698G>A in 3' near gene position were selected and genotyped in 165 Indonesian cancer patients and 188 Caucasian healthy volunteers. Haplotypes were set with gPlink, whereas the difference in haplotype frequencies between Indonesians and Caucasians was compared using multivariate analysis.

Results: The haplotype profiles based on the deletion AAG, 18792A>G and 46698G>A were AAGAA, AAGAG, AAGGG and deletion AG in both of Indonesians and Caucasians. The frequency of the AAGAG haplotype in Indonesians was 54.8% and in Caucasians it was 39.9% ($P < 0.05$). The frequency of AAGGG haplotype in Indonesian was 14.3% and in Caucasians was 29.3%. Moreover, there were significant differences the frequencies of haplotype pairs between Indonesian and Caucasian ($P < 0.001$).

Conclusion: Indonesian cancer patients had significantly different AAGAG and AAGGG haplotype frequencies of the gene encoding the 5-HT3B receptor compared to healthy Caucasians. This finding could be useful for understanding interethnic differences in drug response of drugs targeting the 5-HT3B receptor in cancer treatment related emesis.

INTRODUCTION

The 5-hydroxytryptamine-3 (5-HT3) receptor is a ligand-operated ion channel.[1] This receptor is found in abundance on parasympathetic terminals in the gastrointestinal tract, on the nucleus tractus solitarii and area postrema of central nervous system (CNS).[2] Therefore, this receptors contribute to neuropsychiatric function and the regulation of gastrointestinal functions.[3]

Until now, there are five different receptor subunits of the 5-HT3 receptor in humans: 5-HT3A, B, C, D, and E. The 5-HT3A, B and C are most expressed in the CNS and in the peripheral nervous system, whereas the 5-HT3E receptor is mainly and the 5-HT3D predominantly expressed in the gastrointestinal tract, with the activation of 5-HT3 receptors resulting in various effects, such as emesis and behavioral effects, such as anxiety, depression and cognitive disorders.[4]

Several studies in Caucasians have shown an association between polymorphisms in the gene encoding the 5-HT3B receptor and antiemetic response to 5-HT3 receptor antagonists. 5-HT3 receptor antagonists are frequently used in the prevention and treatment of emesis and vomiting related to drug treatment.[5,6] In addition, some variants of the gene encoding this receptor subtypes have been thought to contribute to neurological and psychiatric disorders.[5] Specifically, the pharmacogenetic studies of genetic variants of the 5-HT3B receptor showed that the deletion AAG at position 100 to 102 was significantly associated with failure of response to 5-HT3B receptor antagonists in chemotherapy-induced nausea and vomiting in Caucasians.[6] In Japanese patients with depressive and anxiety disorders, this variant was associated with paroxetine-induced emesis, next to major depression and etiology of bipolar affective disorders.[6,7] Finally, another study explored possible associations between genetic polymorphisms in nine exons of gene encoding the 5-HTR3B receptor in relation to the occurrence of postoperative nausea and vomiting. This study suggests that 5-HT3B receptor variants may have significant impact on the incidence of postoperative nausea and vomiting.[8]

Today, only limited data are available on haplotypes in the gene encoding the 5-HT3B receptor in Asian population. The aim of this study was therefore to establish allele and haplotype frequencies of the 3 single nucleotide polymorphisms (SNPs) in the gene encoding 5-HTR3B receptor; rs45460698 (deletion AAG in 5'-UTR position), rs4938058 (18792A>G in intron position), and rs7943062 (46698G>A, in 3' near gene position) in Asians and to compare the frequencies with those of a Caucasian population.

METHODS

Population

DNA from 165 Indonesian cancer patients and from 188 Caucasian healthy volunteers was collected for genetic analysis. The Caucasian subjects were men (66%), women (34%) with a mean of age 46.4 ± 13.3 (mean ± SD). However these numbers were calculated from 96 subjects of 188 Caucasian subjects, because the blood donation foundation (Sanquin, The Netherlands) did not provide the characteristics of the remaining donors. The Indonesian cancer patients were recruited at the Sardjito hospital Yogyakarta between January 2009 and November 2009. Most of the cancer patients were women (89.1%) with the diagnoses of cervical cancer (58.8%), ovarian cancer (24.8%), vulva cancer (0.6%) and other cancer (15.8%); the remaining patients were men (10.9%) with a diagnoses of nasopharyngeal cancer. They were first diagnosed as cancer patients at stage I and II (69.1%) and stage III and IV (30.9%). These patients were treated with cisplatin, considered as highly emetogenic chemotherapy and received antiemetics as well. DNA from these subjects was isolated from saliva samples using Oragene™ DNA self-collection kit (DNA Genotek Inc., Ottawa, Ontario, Canada) according to manufacture's prescription. DNA derived from anonymized Caucasian healthy volunteers, was isolated from EDTA anti-coagulated blood using the MagnaPure Compact (Roche Diagnostics, Almere, The Netherlands). The Institutional Review Board of Gadjah Mada University, Yogyakarta, Indonesia approved the study and gave informed consent before enrolment in the study. DNA was quantified using Nanodrop (Isogen, Maarssen, The Netherlands).

Genotypes were established using Taqman assays and analysed on ABI 7500 realtime PCR System from Applied Biosystems (Nieuwerkerk aan den IJssel, The Netherlands) according to the manufacturer's protocol.

Polymorphism selection

Three SNPs in the *5-HT3B* receptor gene, rs45460698 (deletion AAG in 5'-UTR position), rs4938058 (18792A>G in intron position), and rs7943062 (46698G>A, in 3' near gene position) were selected from the National Center for Biotechnology Information (NCBI) SNP database. There were 4 criteria for selection of the SNPs: a minor allele frequency of > 0.2, a validated SNP according to the NCBI database, and preferably a perfect linkage disequilibrium (LD) with other SNPs in *5-HTR3B* receptor gene (D-prime value = 1 and $r^2 \geq 0.7$) and/or indications for relevance based on previous publications.

The SNP rs45460698 was chosen based on the associations with antiemetic response to 5-HT3RAs as shown in previous studies.[8,9] The SNP rs45460698 was chosen based on the associations with antiemetic response to 5-HT3 receptor antagonists as shown in previous studies [8,9]. The SNP rs4938058 is in LD with 6 other SNPs (rs7103572, rs11214769, rs12270070, rs2276307, rs4936285, rs12795805) and the rs7943062 is in LD with the rs11214763 SNP and they were therefore chosen as tagging SNPs. In contrast to the rs45460698 SNP, the rs4938058 and rs7943062 SNPs were not specifically studied in relation to 5-HT3 receptor antagonist response but it is expected that any functional variant in the gene encoding the 5-HT3 receptor will potentially influence the response to the HT3 receptor antagonists and therefore these 2 SNPs were also included in the study.

A challenge in any candidate gene pharmacogenetic study is the selection of polymorphisms. We applied several selection criteria under the assumption that these criteria increased the informative value of the SNP in relation to clinical and functional effects on the gene. However, the *HTR3B* gene is highly polymorphic meaning that our SNPs only represent only a fraction of the possible variation in the population.

5-HT3B receptor gene haplotypes

For the estimation of haplotype frequency and the setting of individual haplotypes from raw genotype data we used the gPlink software with default settings. The estimation of haplotype frequencies/phases was above or equal to 0.01 and phases consideration was above or equal to 0.01 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml, accessed on 22 August 2010).

Statistical analysis

The genotype frequencies were assessed for deviations from Hardy Weinberg equilibrium. The differences in haplotype frequency between Indonesians and Caucasians were compared using multivariate analysis. A P value < 0.05 was considered significant.

RESULTS

The estimated genotype distributions of the three 5-HT3B receptor SNPs in Indonesians and Caucasians are listed in Table 4.1. The genotype distributions did not deviate from Hardy Weinberg equilibrium.

Table 4.1 Percentages of genotypes in the gene encoding the 5-HT3B receptor in Indonesians and Caucasians

	5-HTRB receptor deletion AAG % (n = 349)[#]			5-HTRB receptor 18792A>G % (n = 337)[#]			5-HTRB receptor 46698G>A % (n = 333)[#]		
	AAG-AAG	del- AAG	del-del	AA	GA	GG	GG	GA	AA
Indonesians	69.5	26.8	3.7	74.7	22.2	3.2	74.1	24.7	1.2
Caucasians	76.8	21.6	1.6	53.1	36.9	10.1	68.4	26.9	4.7

[#] The numbers reflect the number of individuals with successful genotype calls.

Table 4.2 Multivariate analysis of 5HT3B receptor haplotypes and ethnicity

Haplotype	*P* value	Odd ratio (Indonesians vs. Caucasians)	95% confidence interval
AAGAG	< 0.001	1.50	1.18-1.91
deletion AG	0.212	1.49	0.72-2.09
AAGGG	< 0.001	0.58	0.43-0.79
AAGAA	0.181	0.81	0.59-1.13

The haplotypes based on LD pattern of the deletion AAG in 5'-UTR, position 18792A>G in intron position and 46698G>A in 3' near gene position were AAGAA, AAGAG, AAGGG and deletion AG in both the Indonesians and Caucasians. Significant differences of haplotype frequencies between Indonesians and Caucasians were found for the AAGAG and AAGGG haplotypes ($P < 0.05$). The frequency of AAGAG haplotype was significantly higher in Indonesians than in Caucasians (54.7% vs 39.4%). The frequency of the AAGGG haplotype in Caucasians was significantly higher than that in Indonesians (29.3% vs 14.3%).

Based on the multivariate analysis, there were significant differences of the haplotype pairs frequencies between Indonesians and Caucasians ($P = 0.001$) (Table 4.2).

Table 4.3 presents the frequencies of haplotype pairs both in Indonesians and Caucasians. AAGAG-AAGAG had the highest haplotype pair frequency percentage (23.9%) and deletion AG-deletion AG the lowest (2.8%).

Table 4.3 Haplotype pairs' frequencies of the gene encoding the 5-HT3B receptor in Indonesians and Caucasians

Haplotype pair	Indonesians *n* (%)	Caucasians *n* (%)	Total *n* (%)
AAGAG-AAGAG	50 (31.8)	28 (16.6)	78 (23.9)
AAGAG-AAGGG	19 (12.1)	34 (20.1)	53 (16.3)
AAGAG-AAGAA	25 (15.9)	27 (16.0)	52 (16.0)
AAGAG-deletion AG	28 (17.8)	18 (10.7)	46 (14.1)
AAGGG-AAGGG	5 (3.2)	18 (10.7)	23 (7.1)
AAGGG-AAGAA	8 (5.1)	15 (8.9)	23 (7.1)
deletion AG-AAGGG	8 (5.1)	14 (8.3)	22 (6.7)
AAGAA-AAGAA	2 (1.3)	8 (4.7)	10 (3.1)
deletion AG-AAGAA	6 (3.8)	4 (2.4)	10 (3.1)
deletion AG-deletion AG	6 (3.8)	3 (1.8)	9 (2.8)

DISCUSSION

To the best of our knowledge, this is the first study to report the haplotypes frequencies in the *5-HT3B* gene in Asians. The study shows that the frequencies of AAGAG and AAGGG haplotypes in the gene encoding 5-HT3B receptor differ significantly between Indonesians and Caucasians. This finding may be helpful to understand interethnic variation of disease and drug response related to the 5-HT3B receptor.[10] We compared allele frequencies in Indonesian cancer patients and Caucasian healthy subjects. To rule out a disease effect, a comparison with Caucasian cancer patients may have been preferable. However, since the study by Tremblay[9] reported comparable genotype frequencies of the insertion/insertion, insertion/deletion and deletion/deletion genotypes of the -100_-102deletion AAG variant (78.1%, 20.7%, 1.2% respectively) in Caucasian cancer patients as we found in Caucasian healthy subjects (76.8%, 21.6%, 1.6% respectively), an effect of disease on allele frequencies is not very likely. In most pharmacogenetic studies regarding the *5-HT3B* receptor gene, the deletion AAG variant was studied for association with drug response phenotypes. In an in vitro study about the functional characterization of the -100_-102 deletion AAG, it was shown that this variant allele affects the 5-HT3B receptor promoter activity by 25-40%, possibly as a result of interference with mRNA.[11] This finding has led to the establishment of an association between this variant and clinical responses.

For example, a study in Caucasians found that patients with the deletion AAG experienced more frequent vomiting after chemotherapy than patients with the other 5-HT3B genotypes. The study result supported that failure of response to 5-HT3 receptor antagonists, such as ondansetron and tropisetron, was related with this deletion of the *5-HT3B* receptor gene.[9] In a second pharmacogenetic study on paroxetine-induced nausea, it was shown that patients with the deletion AAG had significantly more frequent nausea than patients with the homozygous wild type genotype.[6] Yet, in a third study of the influence of 5-HT3B receptor variants to the occurrence of post operative vomiting (POV), it was found that the 100-102 deletion AAG did not have any significant association with the occurrence of POV. However, these authors showed that another deletion variant, the 201-202 deletion of CA, significantly influenced the POV incidence.[8] Likewise, in our study, the frequency of the deletion AAG and the haplotype including the deletion was not significantly different between Indonesians and Caucasians. However, there were significant differences between the Indonesian and Caucasian population in the distribution of the pairs of haplotypes including the deletion AAG. Thus, differences in 5-HT3B antagonist response between Asians and Caucasians can not be ascribed to differences in the frequency of the deletion AAG but may be attributable to the differences in haplotype pairs that exist in each population. However, the effect of the deletion AAG or its haplotype has never been studied in Asian patients in relation to unresponsiveness to 5-HT3 receptor antagonists.

A limitation of this study is that we used self-reported ethnicity. However, to make a more accurate assessment of ethnicity also the ethnicity of the parents and grandparents were verified.

In conclusion, Indonesian cancer patients have significant different haplotypes distribution of gene encoding the 5-HT3B receptor compared to healthy Caucasians. Since previous studies of Tremblay et al.[8] and Rueffert et al.[9] have suggested that the SNP rs45460698 (deletion AAG in 5'-UTR position) was predictive for the efficacy of tropisetron and ondansetron, we propose that the interethnic differences in 5-HT3B haplotype frequencies could result in interethnic differences of response to 5-HT3 receptor antagonists. However, this hypothesis should be tested in a prospective study.

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