EUROPEAN JOURNAL OF CANCER 47 (2011) 1682–1691

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Clinical and genetic factors associated with nausea and vomiting in cancer patients receiving opioids

Eivor A. Laugsand [a,*], *Torill Fladvad* [b], *Frank Skorpen* [b], *Marco Maltoni* [c], *Stein Kaasa* [a,d], *Peter Fayers* [a,e], *Pål Klepstad* [f,g]

[a] *Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), N-7489 Trondheim, Norway*

[b] *Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), N-7489 Trondheim, Norway*

[c] *Palliative Care Unit, Department of Medical Oncology, Morgagni-Pierantoni Hospital, Via Forlanini, 34, 47100 Forli, Italy*

[d] *Department of Oncology, St. Olav University Hospital, N-7006 Trondheim, Norway*

[e] *Department of Public Health, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, United Kingdom*

[f] *Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), N-7489 Trondheim, Norway*

[g] *Department of Anaesthesiology and Emergency Medicine, St. Olav University Hospital, N-7006 Trondheim, Norway*

ARTICLE INFO

Article history:
Available online 12 May 2011

Keywords:
Opioid
Single nucleotide polymorphism
Nausea
Vomiting
Emesis
Adverse effects
Cancer
Palliative care

ABSTRACT

Background: This study investigates whether demographical, disease-related and genetic factors contribute to inter-individual differences in nausea and vomiting among patients receiving opioids for cancer pain.

Methods: Cancer patients receiving opioids were included from 17 centres in 11 European countries. Intensities of nausea and vomiting were reported by 1579 patients on four-point categorical scales. In stratified regression models including demographical and disease-related factors as covariates, 96 single nucleotide polymorphisms (SNPs) in 16 candidate genes related to opioid- or nausea/vomiting signalling pathways (*ABCB1*, *OPRM1*, *OPRK1*, *ARRB2*, *STAT6*, *COMT*, *CHRM3*, *CHRM5*, *HRH1*, *DRD2*, *DRD3*, *TACR1*, *HTR3A*, *HTR3B*, *HTR3C*, *CNR1*) were analysed for association with nausea and vomiting.

Findings: Age, body mass index, Karnofsky Performance Status, gender, use of antiemetics, type of opioid, type of cancer and eight SNPs were associated with the inter-individual differences in nausea and vomiting among cancer patients treated with opioids ($p < 0.01$). The SNPs were rs1176744, rs3782025 and rs1672717 in *HTR3B*; rs165722, rs4680 and rs4633 in *COMT*; rs10802789 and rs685550 in *CHRM3*. Only the SNP rs1672717 in *HTR3B* passed the Benjamini–Hochberg criterion for a 10% false discovery rate.

Interpretation: Clinical characteristics and SNPs within the *HTR3B*, *COMT and CHRM3* genes may be associated with the variability in nausea and vomiting among cancer patients receiving opioids. This knowledge may help to identify patients at particular risk for nausea and vomiting during treatment with opioids for cancer pain.

* *Corresponding author:* Address: Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), P.O. Box 8905, Medisinsk teknisk forskningssenter, N-7491 Trondheim, Norway. Tel.: +47 93021739; fax: +47 73868117.

E-mail address: eivor.a.laugsand@ntnu.no (E.A. Laugsand).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.04.014

1. Introduction

The reported prevalence rates of nausea and vomiting in patients with cancer vary five-fold between studies (nausea 11–78%; vomiting 7–49%).[1] Some of this variability reflects the large inter-individual differences in occurrence and intensity of these symptoms among cancer patients receiving opioids.[2,3] Previous studies have suggested that age,[4,5] gender,[4,5] type of opioid,[6] metastases[3] and cancer diagnoses[3] are associated with the inter-individual differences of nausea and vomiting. Other characteristics such as body mass index, performance status, tumour burden, anticancer treatment,[5] time since initiation of opioids,[2] opioid dose, concurrent disease and use of drugs with anti- or pro-emetic effects may also influence the occurrence and intensity of nausea and vomiting in cancer patients, but have not been thoroughly investigated yet. In addition, recent studies indicate that some of the variability may be associated with genetic variation; either in genes related to opioid action or in genes related to the mechanisms of nausea/vomiting.[2,7–10] However, there is a lack of studies systematically investigating how demographical and disease-related factors contribute to variability in nausea and vomiting among cancer patients receiving opioids, and to our knowledge no studies have addressed the possible contribution from genetic factors.

The effect of opioid analgesics may be influenced by their route of administration, pharmacokinetics, receptor binding, downstream signalling and non-opioid signal-modifying mechanisms. Genes encoding proteins involved in cross-membrane transport of opioids (*ABCB1*), receptor binding and signalling (*OPRM1* and *OPRK1*, *ARRB2*, *STAT6*) or modifying systems (*COMT*) may play a role for the presence and intensity of opioid effects including nausea and vomiting (Fig. 1).[7,11–13]

The signalling pathways of nausea and vomiting, which are separate clinical entities occurring independently or in combination, have recently been reviewed.[3,14,15] Multiple signalling substances and receptors, hence multiple genes (*CHRM3* and *CHRM5*, *HRH1*, *DRD2* and *DRD3*, *TACR1*, *HTR3A*, *HTR3B*, *HTR3C*, *CNR1*) are involved in the four neural pathways converging in the 'vomiting centre' located in the medulla oblongata (Fig. 2).[3,8–10,14–22]

Knowledge of the mechanisms underlying the inter-individual differences in nausea and vomiting during opioid treatment is needed to identify patients at particular risk for nausea/vomiting. Hence, the objective of this study was to investigate, in a clinically relevant cohort of cancer patients receiving opioids, whether the inter-individual differences in nausea and vomiting are associated with demographical factors, disease-related factors or genetic markers represented by single nucleotide polymorphisms (SNPs) of candidate genes in the opioid- or nausea/vomiting signalling pathways.

2. Patients and methods

2.1. Patients

The European Pharmacogenetic Opioid Study (EPOS) is a multicentre, multinational study including 2294 patients from 17 different centres in 11 countries. All patients included were ⩾18 years old, had a verified diagnosis of malignant disease and had received scheduled opioid treatment corresponding to step III at the analgesic ladder of the World Health Organisation (WHO),[23] for at least three days. Patients were excluded if incapable of the language of the study centre. For the genetic association analyses of nausea and vomiting, we also excluded patients receiving chemotherapy ($n = 353$). Non-Caucasians ($n = 47$) and Greek patients ($n = 5$) were excluded to minimise heterogeneity. Finally, patients where no genomic DNA was available ($n = 20$), all genotyping failed ($n = 3$) and neither the question about nausea nor the question about vomiting was answered ($n = 287$) could not be included into the analyses. Thus, the final sample size was 1579 patients from ten countries.

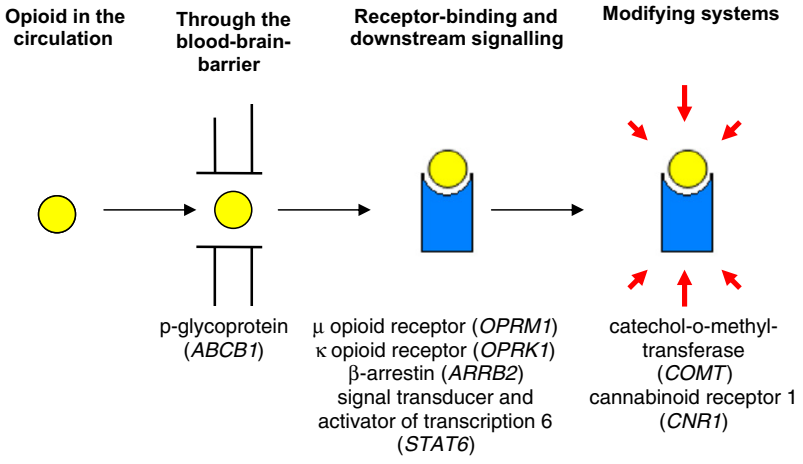

Fig. 1 – A basic illustration of the opioid signalling pathway, also showing the proteins and genes addressed in the present study (genes in parentheses). *Abbreviations:* *ABCB1*, adenosine triphosphate-binding cassette subfamily B member 1gene; *ARRB2*, arrestin β2 gene; *CNR1*, cannabinoid receptor 1 gene; *COMT*, catechol-O-methyltransferase gene; *OPRK1*, opioid receptor κ1 receptor gene; *OPRM1*, opioid receptor μ1 gene; *STAT6*, signal transducer and activator of transcription 6 gene.

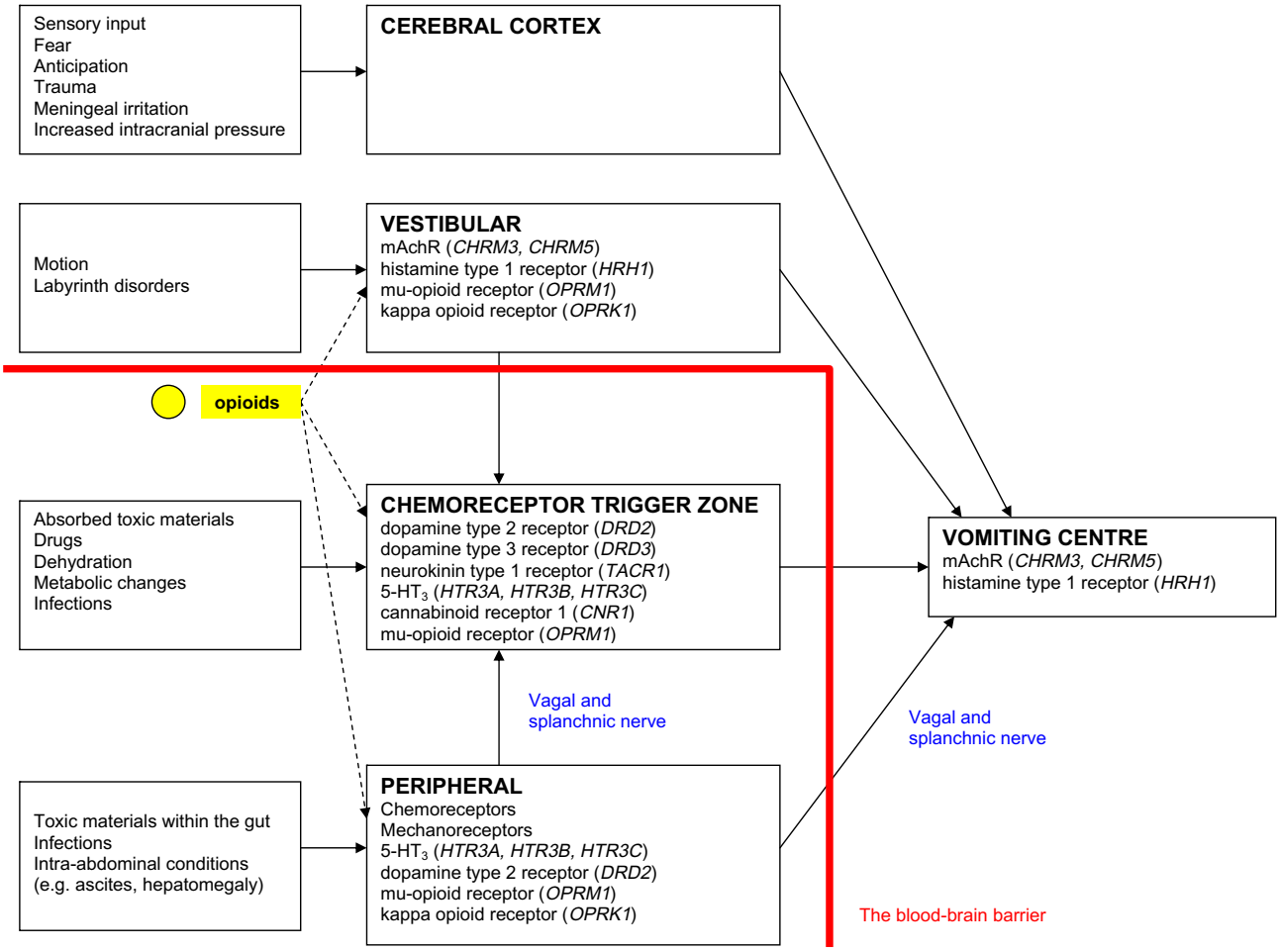

Fig. 2 – A basic illustration of the nausea and vomiting signalling pathways, also showing the proteins and genes addressed in the present study (genes in parentheses). The three mechanisms by which opioids are thought to induce nausea/vomiting are highlighted. *Abbreviations:* *CHRM3*, cholinergic receptor muscarinic 3 gene; *CHRM5*, cholinergic receptor muscarinic 5 gene; *CNR1*, cannabinoid receptor 1 gene; *DRD2*, dopamine receptor D2 gene; *DRD3*, dopamine receptor D3 gene; *HRH1*, histamine type 1 receptor gene; 5-HT$_3$, 5-hydroxytryptamine (serotonin) type 3 receptor; *HTR3A/3B/3C*, 5-hydroxytryptamine (serotonin) receptor 3A/3B/3C gene; mAchR, muscarinic acetylcholine receptor; *OPRK1*, opioid receptor κ1 receptor gene; *OPRM1*, opioid receptor μ1 gene; *TACR1*, tachykinin receptor 1 gene.

The study was designed and performed in accordance with the Declaration of Helsinki. Ethical committees at each study centre or in each country approved the study. All patients gave written informed consent before inclusion.

2.2. *Assessments*

Patients reported nausea and vomiting by answering the European Organisation for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC-QLQ-C30).[24] Symptom intensity was assessed by four-point verbal rating scales with the notations 'not at all, a little, quite a bit and very much'. Age, gender, body mass index (BMI), time since start of opioid treatment, cancer diagnoses and localisation of metastases were registered by a health care provider (physician or nurse). Providers also registered the consumption of all drugs, country, Karnofsky Performance Status (KPS),[25] and cognitive function by the Mini Mental State (MMS) Examination.[26]

2.3. *SNP selection, genotyping and quality control*

Based on associations identified from the literature, putative effect on nausea/vomiting, available information in databases (SNPper, dbSNP, Ensembl, HapMap), and the frequency, functionality and distance between SNPs, 104 SNPs within 16 candidate genes were selected (Supplementary Table 1). With a few exceptions for SNPs identified as important in the literature, all selected SNPs had an expected minor allele frequency (MAF) of $\geqslant$10% in Caucasians. All SNPs were present and compatible with assay rules in the software SNPbrowser version 3.5 (Applied Biosystems).

Genomic DNA was isolated from EDTA whole blood using the Gentra Puregene blood kit (QIAGEN Science, Maryland, USA) at HUNT Biobank, Levanger, Norway. Genotyping was performed by applying the SNPlex Genotyping System according to the supplier's dry DNA protocol, using universal SNPlex System kits and reagents and SNP-specific ligation probes (Applied Biosciences, Foster City, CA, USA). The SNPlex

signals were analysed using the GeneMapper® Software v4.0 (Applied Biosciences, Foster City, CA, USA) followed by manual reading, quality control and data cleaning. Samples giving low signals inseparable from negative controls and SNPs with inconsistent clustering on inspection were rejected prior to analysis and treated as missing data.

2.4. Statistical analysis

The possible associations with demographical and disease-related factors were investigated by univariate and multivariate linear regressions with the EORTC nausea/vomiting scale as the outcome of interest. The factors explored were age, BMI, KPS, time on opioids, total daily oral morphine equivalent dose, gender, use of antiemetics in the past 24 h (yes/no), use of corticosteroids in the past 24 h (yes/no), type of opioid, metastases and cancer diagnosis. Age, BMI and KPS were analysed both as continuous and as dichotomised variables (age $\leqslant$60 versus >60,[5] BMI <25 versus $\geqslant$25, KPS $\leqslant$80 versus >80[27]). All factors significantly ($p < 0.05$) associated with nausea/vomiting in univariate analyses were included in the stepwise multivariate analysis stratified by country.

Genotype and allele frequencies were determined and quality checked. SNPs where no genotypes were recorded, SNPs where genotypes were not in Hardy–Weinberg equilibrium (HWE) (Chi-squared test, $p < 0.0005$) and SNPs with an observed MAF <5% were rejected. The primary outcome was the EORTC QLQ-C30 combined nausea/vomiting scale (range 0–100) for which multivariate linear regression was used. In addition, two separate multivariate ordered logistic regressions were performed for the nausea item (range 1–4) and the vomiting item (range 1–4). All regressions were stratified by country and use of antiemetics was included as a binary stratification variable (antiemetics used/not used) for each country.[28] The demographical and disease-related factors associated with nausea/vomiting ($p < 0.05$) were entered as covariates. Analyses were also repeated without the inclusion of covariates, as a sensitivity check. Unstratified analyses not including covariates were used to compare symptom intensity between those carrying the 'risk' allele and those not. Approaches adopted to mitigate the multiplicity issues were the use of a 10% false discovery rate (FDR) reporting the Benjamini–Hochberg (BH) thresholds,[29] the pre-specification of the nausea/vomiting scale as the primary outcome and the pre-specification of the codominant genetic model for the primary analyses (dominant, recessive and additive models were exploratory). We evaluated the data on an exploratory basis, by interpreting p-values <0.01 as suggestive of effects that should be evaluated in future studies. STATA version 11.0 was used for all analyses (StataCorp. 2009 *STATA Statistical Software: Release 11*. College Station, TX: StataCorp LP).

3. Results

3.1. Patients

Among the 1579 patients included in the analyses, mean age was 62 years, mean KPS was 61 and mean MMS total score was 27. Fifty-four percent of patients were men, 79% were hospitalised and 83% had one or more metastases. The number of patients per country varied from 25 (Finland) to 405 (Norway). 'Quite a bit' or 'very much' nausea was reported by 27% of patients and 14% reported 'quite a bit' or 'very much' vomiting. Further characteristics of patients and opioid treatment are given in Table 1.

3.2. Association with demographical and disease-related factors

The reported intensity of nausea and vomiting was significantly ($p < 0.05$) associated with age, BMI, KPS, gender, use of antiemetics, use of steroids, type of opioid, metastasis and cancer diagnosis (Table 2) in univariate analyses. The distribution of nausea and vomiting for each country is given in Supplementary Table 2. In multivariate analyses stratified by country, lower age, lower BMI, lower KPS ($\leqslant$80), female gender, use of antiemetics, use of other opioids than fentanyl, and cancer of the female reproductive organs were associated with more nausea and vomiting ($p < 0.05$) (Table 2). Thus, these factors were included as covariates in the multivariate regressions of genetic factors. No clear evidence of any important interaction effects between covariates were found (data not shown), thus subsequent analyses did not allow for such interactions.

3.3. Genotype distributions

The genotyping success rates, genotype- and allele frequencies are shown in Supplementary Table 1. Three SNPs (rs1202181 in *ABCB1*, rs7175823 in *CHRM5* and rs33940208 in *HTR3A*) were excluded from analyses because no genotypes were recorded. All remaining SNPs were in Hardy–Weinberg equilibrium. Five SNPs (rs7815824 in *OPRK1*, rs16954146 in *ARRB2*, rs1800496 in *DRD2*, rs34327364 in *HTR3A* and rs3831455 in *HTR3B*) were excluded from subsequent analyses due to MAF <5% and 96 SNPs were retained for further analyses.

3.4. Association with genetic factors

Within the *HTR3B* gene, associations between two SNPs (rs1176744 and rs1672717) and the nausea/vomiting scale (Table 3), three SNPs (rs1176744, rs3782025 and rs1672717) and the intensity of nausea (Table 4), and the SNP rs1176744 and vomiting (Table 5) were suggested. The significant association between rs1672717 and nausea ($p = 0.0002$) in a dominant model passed the BH-threshold (Table 4). Patients carrying the G-allele of rs1176744, the T-allele of rs3782025 and the T-allele of rs1672717 had less nausea/vomiting (Table 3–5).

Two SNPs within the *COMT* gene (rs165722 and rs4680) were associated with the intensity of nausea and vomiting (Table 3) and nausea (Table 4) in codominant models ($p < 0.01$). In addition, the findings indicated an association between rs4633 and the nausea/vomiting scale (Table 3) in a dominant model ($p < 0.01$), but this SNP was successfully genotyped in only 462 patients (Supplementary Table 1). None of these associations passed the BH-criterion. Patients carrying the C-allele of rs165722, the T-allele of rs4633 and the G-allele of rs4680 had less nausea/vomiting (Table 3 and 4).

Table 1 – Patient demographics, antiemetic treatment, intensity of nausea and vomiting (N = 1579).

	Mean	SD
Age (years)	61.9	12.0
Body mass index (kg/m^2)	23.6	4.6
Karnofsky performance status (range 0–100)	61.1	16.5
Mini mental state, total score (range 0–30)	26.9	3.3
Time since diagnosis (months)	30.8	44.6
Creatinine serum concentrations (μmol/l)	74.4	39.8
Albumin (g/l)	31.7	7.1
EORTC NAUSEA/VOMITING scale (range 0–100)	23.8	28.3
	N	%
Gender		
Female	729	46.2
Male	850	53.8
Department		
Hospitalised patients	1254	79.4
Out-patients	325	20.6
Status of opioid treatment		
Opioid recently initiated/titration	301	19.1
Stable dosing	1266	80.2
Antiemetic during the past 24 h		
Yes	605	38.3
No	974	61.7
Systemic steroid during the past 24 h		
Yes	759	48.1
No	820	51.9
Metastasis		
Bone	691	43.8
CNS	84	5.3
Liver	383	24.3
Lung	345	21.8
Other	635	40.2
None	274	17.4
Cancer diagnosis		
Gastrointestinal (including liver, pancreas)	373	23.6
Female reproductive organs	129	8.2
Head and neck	96	6.1
Other	981	62.1
Type of opioid		
Morphine	622	39.4
Oxycodone	336	21.3
Fentanyl	454	28.8
Other	167	10.5
Country		
Denmark	28	1.8
Finland	25	1.6
Germany	240	15.2
Iceland	115	7.3
Italy	308	19.5
Lithuania	41	2.6
Norway	405	25.6
Sweden	107	6.8
Switzerland	88	5.6
United Kingdom	222	14.1
EORTC 14 NAUSEA		
Not at all	706	44.7
A little	438	27.7
Quite a bit	274	17.4
Very much	157	9.9
EORTC 15 VOMITING		
Not at all	1077	68.2
A little	281	17.8
Quite a bit	133	8.4
Very much	83	5.3

Abbreviation: SD, standard deviation.

Within *CHRM3*, associations were denoted between the T-allele of rs685550 and more nausea/vomiting in a dominant model (Table 3), and between the T-allele of rs10802789 and more nausea in a codominant model (Table 4). These associations did not pass the BH-criterion.

4. Discussion

Among cancer patients treated with opioids, the intensity of nausea and vomiting was significantly associated with age, BMI, KPS, gender, use of antiemetics, type of opioid and type of cancer. In addition, this exploratory study of 96 SNPs points to *HTR3B*, *COMT* and *CHRM3* as candidate genes for contribution to the inter-individual differences in nausea and vomiting. Among the eight SNPs associated, only the association with rs1672717 in *HTR3B* persisted after correcting for multiple tests.

The demographical and disease-related characteristics of included patients were as expected for cancer patients.[1,30] The prevalence of moderate or severe nausea and vomiting (27% and 14%), and the use of antiemetics (38%) agree with previous reports.[1,30] In agreement with clinical experience and the existing literature, we found that lower age,[4,5] deteriorating physical status (lower BMI, lower KPS),[27] female gender or cancer of the female reproductive organs,[4,5] and the need for antiemetics were associated with more nausea/vomiting. Nausea and vomiting were also associated with use of opioids other than transdermal fentanyl.[6] Severity of nausea and vomiting also differed between countries, possibly reflecting different admittance-criteria of the centres, cultural or linguistic differences of symptom assessment although considered less important for nausea and vomiting,[31] or differences in clinical practise regarding symptomatic treatment.[30]

Our findings suggest that SNPs within *HTR3B* may contribute to nausea/vomiting. Activation of 5-HT$_3$ receptors in the gastrointestinal-tract or in the chemoreceptor trigger zone may lead to nausea and vomiting.[3] Of the five human receptor subunits (5-HT$_{3A–E}$),[20,21] the 5-HT$_{3B}$ subunit is considered a major determinant of serotonin-receptor function. In the present study, patients carrying the G-allele (129Ser) of rs1176744 (Tyr129Ser, c.386A > C) had less intense nausea and vomiting. This finding is in line with the significantly higher risk of paroxetine-induced nausea identified in Tyr/Tyr carriers[17] and the trend towards increased risk of chemotherapy-induced vomiting in Tyr/Tyr carriers,[9] whereas no

Table 2 – Demographical and disease-related factors associated with the EORTC QLQ-C30 nausea/vomiting-scale.

	Mean	SD	Univariate analysis			Multivariate analysis		
			β	95% CI	P	β	95% CI	P
Age (years)			−0.16	−0.28 to −0.05	0.006	−0.15	−0.26 to −0.04	0.006
≤60 (0)	25.4	29.0						
>60 (1)	22.6	27.7	−2.98	−5.80 to −0.16	0.038			
BMI (kg/m²)			−0.33	−0.64 to −0.02	0.034	−0.28	−0.56 to −0.01	0.045
<25 (0)	24.3	28.6						
≥25 (1)	22.1	27.4	−2.32	−5.32 to 0.68	0.129			
KPS (range 0–100)			−0.07	−0.16 to 0.01	0.106			
≤80 (0)	24.2	28.5						
>80 (1)	16.9	22.9	−7.43	−13.57 to −1.29	0.018	−5.78	−10.48 to −1.07	0.016
Time since start opioids								
≤2 weeks (0)	26.2	28.8						
>2 weeks (1)	22.6	27.9	−2.40	−5.82 to 1.02	0.169			
Total daily oral morphine equivalent dose in mg								
≤300 mg (0)	24.2	28.3						
>300 mg (1)	23.1	28.2	0.49	−0.73 to 1.72	0.431			
Gender								
Male (0)	21.1	26.2						
Female (1)	26.9	30.3	5.83	3.03 to 8.62	<0.001	3.12	0.25 to 5.98	0.033
Antiemetic								
Yes (0)	34.3	31.2						
No (1)	17.3	24.1	−17.03	−19.78 to −14.28	<0.001	−16.17	−19.10 to −13.24	<0.001
Steroids								
Yes (0)	21.0	27.2						
No (1)	26.4	29.0	5.47	2.68 to 8.26	<0.001			
Type of opioid (0 = no, 1 = yes)								
Morphine	23.6	27.9	−1.41	−4.30 to 1.47	0.336			
Oxycodone	25.3	27.5	2.76	−0.75 to 6.28	0.124			
Fentanyl	22.4	28.6	−5.18	−8.89 to −1.47	0.006	−5.26	−8.68 to −1.85	0.003
Metastases (0 = no, 1 = yes)								
Bone	22.4	28.1	−2.54	−5.36 to 0.28	0.077			
CNS	25.6	29.3	1.81	−4.41 to 8.03	0.569			
Liver	25.8	29.4	2.66	−0.61 to 5.93	0.111			
Lung	23.6	29.3	−0.22	−3.61 to 3.17	0.898			
Other	25.6	29.7	3.06	0.21 to 5.91	0.036			
Cancer diagnosis (0 = no, 1 = yes)								
Gastrointestinal	22.3	28.8	−1.78	−5.08 to 1.51	0.289			
Female reproductive organs	33.5	32.0	11.14	6.05 to 16.22	<0.001	7.83	1.96 to 13.70	0.009
Head and neck	25.4	30.2	1.74	−4.10 to 7.58	0.559			
Other	22.9	27.6	−2.38	−5.30 to 0.54	0.110			

Note: Both univariate and multivariate analyses were stratified by country. Age, BMI and KPS were investigated in the univariate analyses both as continuous variables and as dichotomous variables. The logarithm of the total opioid oral morphine equivalent dose in milligrams was used for the univariate analysis.

Abbreviations: SD, standard deviation; CI, confidence interval; BMI, body mass index; KPS, Karnofsky performance status.

association was found between rs1176744 and postoperative vomiting[18] or fluvoxamine-induced nausea.[19] The functional consequences of the amino acid substitution (Tyr129Ser) in the extracellular N-terminal domain,[20] are 20- to 10-fold slower deactivation and desensitisation kinetics of the altered receptor (129Ser).[32] The altered receptor function might lead to an altered activity of vagal afferent neurons and neurons located in the dorsal vagal complex of the brainstem, leading to a decreased susceptibility towards nausea and vomiting. Although the functionality of the intronic SNPs rs3782025 (c.696 + 3792G > A) and rs1672717 (c.697–971G > A) are unclear, the strength of associations between SNPs within the gene and nausea/vomiting is likely to be similar as *HTR3B* is covered by only one haploblock in Caucasians (HapMap). The present and previous studies[9,17] altogether indicate that genetic variants within *HTR3B* could be of clinical relevance for treatment of nausea/vomiting. Studies on nausea/vomiting of different aetiologies, mapping the response to 5-HT_3 antagonists in carriers of the altered genetic variants, are needed.

COMT polymorphisms may influence nausea and vomiting as the catechol-O-methyltransferase (COMT) enzyme

Table 3 – Genetic factors associated with the EORTC QLQ-C30 nausea/vomiting scale (N = 1579[a]).

Gene	Genotype	Scale	Multivariate analysis					*p*-Value alleles**
SNP	*Allele*	Mean	SD	*β*	95% CI	*P**	Model	
HTR3B								
rs1176744	GG	24.8	28.9					
	GT	21.7	27.4	4.08	1.20 to 6.96	0.005	Codominant	
	TT	26.3	29.2	4.00	1.09 to 6.91	0.007	Recessive	
	G	22.2	27.7					0.004
	Not G	26.3	29.2					
rs1672717	CC	28.6	28.8					
	CT	22.9	27.9	5.81	1.82 to 9.80	0.004	Dominant	
	TT	23.6	28.7					
	T	23.2	16.7					0.002
	Not T	28.6	28.8					
COMT								
rs165722	CC	26.7	30.7					
	CT	21.7	26.7	4.99	1.97 to 8.02	0.001	Codominant	
	TT	26.8	29.3					
	C	23.2	28.1					0.045
	Not C	26.8	29.3					
rs4680	AA	26.2	29.2					
	AG	21.5	26.6	4.39	1.63 to 7.14	0.002	Codominant	
	GG	25.8	30.5					
	G	22.8	27.9					0.030
	Not G	26.2	29.2					
rs4633	CC	36.4	32.1					
	CT	25.3	28.7	8.88	2.30 to 15.46	0.008	Dominant	
	TT	29.9	29.2					
	T	27.0	28.9					0.006
	Not T	36.4	32.1					
CHRM3								
rs685550	CC	18.0	22.5					
	CT	25.4	28.9	−6.74	−11.60 to −1.88	0.006	Dominant	
	TT	24.3	28.9					
	T	24.7	28.9					0.065
	Not T	18.0	22.5					

Abbreviations: SNP, single nucleotide polymorphism; SD, standard deviation; CI, confidence interval.

[a] In 9 patients where one of the two EORTC QLQ-C30 questions (14 or 15) was missing, the other item was used to estimate the nausea/vomiting score.

* *p*-Value of ordinal logistic regression in the analyses allowing for covariates and stratified by country and use of antiemetics.

** *p*-Value of unstratified analyses without the inclusion of covariates.

modulates neurotransmission by metabolising the catecholamine dopamine. Dopamine D_2 receptor blockade in the area postrema and vomiting centre have an antiemetic effect and enhanced dopaminergic activity in patients receiving COMT inhibitors leads to more nausea/vomiting. Increased dopaminergic neurotransmission in individuals with a low-activity COMT enzyme, cause reduced levels of enkephalins and higher density of μ opioid receptors in the brain, thereby influencing pain, analgesia and probably other opioid effects.[33] There are no details about clinical relevance or functionality of the intronic rs165722 (c.−1 + 201C > T). The SNP rs4680 (c.472G > A) is a missense mutation leading to an amino acid exchange (Val158Met) and a three-to four-fold reduction of the COMT enzyme activity (158Met).[33] In migraine without aura, patients with the Met-allele had an increased incidence of nausea/vomiting, most likely due to the elevated levels of dopamine.[34] In line with this hypothesis, patients carrying the G-allele reported less intense symptoms in the present study. As the T-allele of the rs4633 synonymous mutation (His62His, c.186C > T) is closely linked with the A-allele of rs4680 (HapMap), and the TT genotype has been associated with reduced expression levels of COMT enzyme,[33] we anticipated that the TT genotype would be associated with elevated levels of dopamine and increased nausea/vomiting. However somewhat unexpected, patients carrying the T-allele reported less nausea and vomiting in the present study. One conceivable explanation for this discrepancy is that only 462 patients were successfully genotyped for the rs4633 polymorphism, possibly leading to a skewed genotype association. Rakvåg et al. did not find any association between SNPs in *COMT* and nausea/vomiting,[7,35] but these studies were not primarily designed to detect such associations, the sample sizes were small (*N* = 207 and *N* = 197) and the use of antiemetics was not controlled for in the analyses.

The results also indicated possible associations between SNPs in *CHRM3* (rs685550 (c.−146–66144G > A) and rs10802789 (c.−249–8806C > T)) and nausea/vomiting. The

Table 4 – Genetic factors associated with nausea (N = 1575).

Gene	Genotype	Absolute number of patients					Multivariate analysis				p-Value **
SNP	*Allele*	Not at all	A little	Quite a bit	Very much	Total	OR	95% CI	P*	Model	
HTR3B											
rs1176744	GG	59	44	23	16	142					
	GT	327	174	109	61	671	1.31	1.08 to 1.59	0.007	Codominant	
	TT	260	189	121	70	640					
	G	*386*	*218*	*132*	*77*	*813*					*0.012*
	Not G	*260*	*189*	*121*	*70*	*640*					
rs3782025	CC	103	82	59	28	272					
	CT	302	193	107	72	674	1.41	1.10 to 1.79	0.006	Dominant	
	TT	158	80	56	33	327					
	T	*460*	*273*	*163*	*105*	*1001*					*0.029*
	Not T	*103*	*82*	*59*	*28*	*272*					
rs1672717	CC	77	74	50	26	227					
	CT	344	202	114	75	735	1.59	1.24 to 2.04	**0.0002**	Dominant	
	TT	244	145	98	49	536					
	T	*588*	*347*	*212*	*124*	*1271*					*0.001*
	Not T	*77*	*74*	*50*	*26*	*227*					
COMT											
rs165722	CC	127	76	56	36	295					
	CT	307	201	102	54	664	1.35	1.10 to 1.65	0.004	Codominant	
	TT	140	91	74	42	347					
	C	*434*	*277*	*158*	*90*	*959*					*0.022*
	Not C	*140*	*91*	*74*	*42*	*347*					
rs4680	AA	175	114	87	51	427					
	AG	373	232	119	63	787	1.33	1.10 to 1.60	0.002	Codominant	
	GG	152	86	61	42	341					
	G	*525*	*318*	*180*	*105*	*1128*					*0.010*
	Not G	*175*	*114*	*87*	*51*	*427*					
CHRM3											
rs10802789	CC	200	103	65	33	401					
	CT	256	180	111	75	622	0.74	0.59 to 0.92	0.007	Codominant	
	TT	106	67	49	25	247					
	T	*362*	*247*	*160*	*100*	*869*					*0.005*
	Not T	*200*	*103*	*65*	*33*	*401*					

Abbreviation: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

* *p*-Value of ordinal logistic regression in the analyses allowing for covariates and stratified by country and use of antiemetics. *p*-Values in **bold** represent associations that passed the Benjamini–Hochberg criterion for selection required by a 10% false discovery rate correction for multiple testing.

** *p*-Value of unstratified analyses without the inclusion of covariates.

Table 5 – Genetic factors associated with vomiting (N = 1574).

Gene	Genotype	Absolute number of patients					Multivariate analysis				p-Value alleles**
SNP	*Allele*	Not at all	A little	Quite a bit	Very much	Total	OR	95% CI	P*	Model	
HTR3B											
rs1176744	GG	99	21	15	8	143					
	GT	477	119	39	34	669	1.35	1.09 to 1.69	0.007	Recessive	
	TT	413	121	70	36	640					
	G	*576*	*140*	*54*	*42*	*812*					*0.007*
	Not G	*413*	*121*	*70*	*36*	*640*					

Abbreviation: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

* *p*-Values of ordinal logistic regression in the analyses allowing for covariates and stratified by country and use of antiemetics.

** *p*-Value of unstratified analyses without the inclusion of covariates.

functional consequences of these SNPs are unknown. However, the muscarinic acetylcholine receptors including the M_3 encoded by *CHRM3*, have been associated with the emetic pathway and opioid-induced nausea/vomiting,[15] and M_3 muscarinic antagonists impede motion sickness and opioid-induced nausea/vomiting.[16]

Previous findings of associations between nausea/vomiting and genetic variants within *OPRM1*,[11] *DRD2*,[8] *HTR3B*,[9]

HTR3C,[10] *ABCB1*,[12] *ARRB2* or *STAT6* genes[13] were not replicated. This may reflect the lack of reproducibility hampering genetic association studies in general. Smaller studies, often without strategies for control of covariates or handling of multiple analyses readily result in non-replicable associations.

We recognise some strengths and limitations of this study. First, there may be several possible definitions of the nausea/vomiting-phenotype in cancer patients receiving opioids and antiemetics. The use of non-validated or site specific tools to assess nausea/vomiting precludes comparison of results between studies. A strength of this study is that we used the EORTC QLQ-C30, a well-validated assessment tool which is formally translated into many languages. Other features in favour of this study are that the number of patients is higher than in other studies addressing genetic variability related to opioid effects, that measures have been undertaken to control for false positive findings, that more than one or a few candidate SNPs are included into the analyses and, finally, that potential clinical confounding factors are identified and included into the analytical strategy. A limitation of the study is that symptom intensity was recorded over the past week, whereas administration of antiemetics was recorded over the past 24 h. However, we believe that since the use of antiemetics was recorded for the last period of the assessment interval, the use of antiemetics is related to the symptom intensity. Moreover, the symptom assessments for the last 24 h and the past week are closely related in cancer patients.[36] In the present as in other genetic studies selecting candidate genes, the outcome can be related to other hereto unexplored genes. New studies investigating other genes or using other analytical techniques, such as genome wide association studies, may identify genetic variation important for nausea and vomiting. For instance, Hammer et al. observed that the SNP rs6443930 in *HTR3D* was associated with chemotherapy-induced vomiting after the analyses of the present study was performed.[37] Whether this variant is important in patients receiving opioids for cancer pain is unknown. Finally, another limitation is that no replication sample was included. Therefore, the findings in this study should be replicated in a technically and biologically independent study before the associations can be regarded as conclusive.

In summary, the inter-individual variations in nausea and vomiting seen among cancer patients receiving opioids are associated with age, BMI, KPS, gender, use of antiemetics, type of opioid and type of cancer. In addition, single nucleotide polymorphisms within the *HTR3B, COMT and CHRM3* genes may play a significant role. This knowledge may help clinicians to identify patients at particular risk for nausea and vomiting during opioid treatment for cancer pain, thereby improving the evidence base for individualised treatment and avoidance of unnecessary patient suffering.

Sources of support

This study was supported by grants from the European Palliative Care Research Collaborative (EPCRC), the European Commission's Sixth Framework Programme, the Norwegian University of Science and Technology and the Research Council of Norway.

Author contributions

EAL, FS, SK and PK were responsible for the study conception and design. EAL, TF, FS, MM, SK and PK contributed to the acquisition of data. EAL, TF, PF and PK analysed and interpreted the data. EAL and PK were responsible for drafting of the manuscript. All authors critically revised the manuscript.

Conflict of interest statement

EAL: No conflicts of interest and declare as the corresponding author that she had full access to all the data in the study and had final responsibility for the decision to submit for publication.

TF: None

FS: one honorary from Wyeth (now Pfizer) to disclose.

MM: None

SK: discloses research funding from Nycomed and payment for lectures from Nycomed and Pfizer outside the submitted work. SK also received royalties for the Oxford Textbook of Palliative Medicine.

PF: None

PK: discloses advisory board membership for Orion Pharma, lecture honorarium from Mundipharma Wyeth, travel/accommodation expenses from Pfizer and Wyeth outside the submitted work.

Acknowledgements

The authors are grateful to all the researchers involved in The European Pharmacogenetic Opioid Study (EPOS).

This study was supported by grants from the European Palliative Care Research Collaborative (EPCRC), the European Commission's Sixth Framework Programme, the Norwegian University of Science and Technology and the Research Council of Norway. The funding sources were not involved in study design, in collection, analysis or interpretation of data, in writing of the report or in the decision to submit the paper for publication.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.04.014.

REFERENCES

1. Teunissen SCCM, Wesker W, Kruitwagen C, et al. Symptom prevalence in patients with incurable cancer: A systematic review. *J Pain Symptom Manag* 2007;**34**:94–104.
2. Cherny N, Ripamonti C, Pereira J, et al. Strategies to manage the adverse effects of oral morphine: an evidence-based report. *J Clin Oncol* 2001;**19**:2542–54.

3. Wood GJ, Shega JW, Lynch B, Von Roenn JH. Management of intractable nausea and vomiting in patients at the end of life – 'I was feeling nauseous all of the time, nothing was working'. *JAMA* 2007;**298**:1196–207.
4. Walsh D, Donnelly S, Rybicki L. The symptoms of advanced cancer: relationship to age, gender, and performance status in 1000 patients. *Support Care Cancer* 2000;**8**:175–9.
5. Foubert J, Vaessen G. Nausea: the neglected symptom? *Eur J Oncol Nurs* 2005;**9**:21–32.
6. Ahmedzai S, Brooks D. Transdermal fentanyl versus sustained-release oral morphine in cancer pain: preference, efficacy, and quality of life. The TTS-Fentanyl Comparative Trial Group. *J Pain Symptom Manag* 1997;**13**:254–61.
7. Skorpen F, Laugsand EA, Klepstad P, Kaasa S. Variable response to opioid treatment: any genetic predictors within sight? *Palliat Med* 2008;**22**:310–27.
8. Ribeiro-Dasilva M, Wallace M, Glover T. The dopamine receptor 2 (DRD2) gene is associated with opioid-induced nausea and emesis. *J Pain* 2009;**10**(suppl1):S29.
9. Tremblay PB, Kaiser R, Sezer O, et al. Variations in the 5-hydroxytryptamine type 3B receptor gene as predictors of the efficacy of antiemetic treatment in cancer patients. *J Clin Oncol* 2003;**21**:2147–55.
10. Fasching PA, Kollmannsberger B, Strissel PL, et al. Polymorphisms in the novel serotonin receptor subunit gene HTR3C show different risks for acute chemotherapy-induced vomiting after anthracycline chemotherapy. *J Cancer Res Clin Oncol* 2008;**134**:1079–86.
11. Skarke C, Darimont J, Schmidt H, Geisslinger G, Lotsch J. Analgesic effects of morphine and morphine-6-glucuronide in a transcutaneous electrical pain model in healthy volunteers. *Clin Pharmacol Ther* 2003;**73**:107–21.
12. Coulbault L, Beaussier M, Verstuyft C, et al. Environmental and genetic factors associated with morphine response in the postoperative period. *Clin Pharmacol Ther* 2006;**79**:316–24.
13. Ross JR, Rutter D, Welsh K, et al. Clinical response to morphine in cancer patients and genetic variation in candidate genes. *Pharmacogenomics J* 2005;**5**:324–36.
14. Hornby PJ. Central neurocircuitry associated with emesis. *Am J Med* 2001;**111**(suppl 8A):106S–12.
15. Sanger GJ, Andrews PL. Treatment of nausea and vomiting: gaps in our knowledge. *Auton Neurosci* 2006;**129**:3–16.
16. Kotelko DM, Rottman RL, Wright WC, et al. Transdermal scopolamine decreases nausea and vomiting following cesarean section in patients receiving epidural morphine. *Anesthesiology* 1989;**71**:675–8.
17. Sugai T, Suzuki Y, Sawamura K, et al. The effect of 5-hydroxytryptamine 3A and 3B receptor genes on nausea induced by paroxetine. *Pharmacogenomics* 2006;**J6**:351–6.
18. Rueffert H, Thieme V, Wallenborn J, et al. Do Variations in the 5-HT3A and 5-HT3B Serotonin Receptor Genes (HTR3A and HTR3B) Influence the Occurrence of Postoperative Vomiting? *Anesth Analg* 2009;**109**:1442–7.
19. Suzuki Y, Sawamura K, Someya T. Polymorphisms in the 5-hydroxytryptamine 2A receptor and cytochromeP4502D6 genes synergistically predict fluvoxamine-induced side effects in Japanese depressed patients. *Neuropsychopharmacology* 2006;**31**:825–31.
20. Walstab J, Hammer C, Bonisch H, Rappold G, Niesler B. Naturally occurring variants in the HTR3B gene significantly alter properties of human heteromeric 5-hydroxytryptamine-3A/B receptors. *Pharmacogenet Genomics* 2008;**18**:793–802.
21. Niesler B, Walstab J, Combrink S, et al. Characterization of the Novel Human Serotonin Receptor Subunits 5-HT3C, 5-HT3D, and 5-HT3E. *Mol Pharmacol* 2007;**72**:8–17.
22. Tanaka M, Kobayashi D, Murakami Y, et al. Genetic polymorphisms in the 5-hydroxytryptamine type 3B receptor gene and paroxetine-induced nausea. *Int J Neuropsychopharmacol* 2008;**11**:261–7.
23. WHO. *Cancer pain relief*. 2nd ed. Geneva: World Health Organisation; 1996.
24. Aaronson N, Ahmedzai S, Bergman B, et al. The European Organization for Research and Treatment of Cancer QLQ-C30 – A quality-of-life instrument for use in international clinical trials in oncology. *J Natl Cancer Inst* 1993;**85**:365–76.
25. Karnofsky D, Abelmann W, Craver L, Burchenal JH. The use of nitrogen mustard in the palliative treatment of cancer. *Cancer* 1948;**1**:634–56.
26. Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;**12**:189–98.
27. Portenoy RK, Thaler HT, Kornblith AB, et al. Symptom prevalence, characteristics and distress in a cancer population. *Qual Life Res* 1994;**3**:183–9.
28. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med* 2005;**24**:2911–35.
29. Benjamini Y, Hochberg Y. On the adaptive control of the false discovery fate in multiple testing with independent statistics. *J Educ Behav Stat* 2000;**25**:60–83.
30. Laugsand E, Kaasa S, de Conno F, Hanks G, Klepstad P. Intensity and treatment of symptoms in 3030 palliative care patients: a cross-sectional survey of the EAPC Research Network. *J Opioid Manag* 2009;**5**:11–21.
31. Scott NW, Fayers PM, Aaronson NK, et al. The use of differential item functioning analyses to identify cultural differences in responses to the EORTC QLQ-C30. *Qual Life Res* 2007;**16**:115–29.
32. Krzywkowski K, Davies P, Feinberg-Zadek P, Brauner-Osborne H, Jensen A. High-frequency HTR3B variant associated with major depression dramatically augments the signaling of the human 5-HT3AB receptor. *Proc Natl Acad Sci USA* 2008;**105**:722–7.
33. Andersen S, Skorpen F. Variation in the COMT gene: implications for pain perception and pain treatment. *Pharmacogenomics* 2009;**10**:669–84.
34. Park JW, Lee KS, Kim JS, Kim YI, Shin HE. Genetic contribution of catechol-O-methyltransferase polymorphism in patients with migraine without aura. *J Clin Neurol* 2007;**3**:24–30.
35. Rakvåg TT, Ross JR, Sato H, et al. Genetic variation in the catechol-O-methyltransferase (COMT) gene and morphine requirements in cancer patients with pain. *Mol Pain* 2008;**4**:64.
36. Shi QL, Trask PC, Wang XS, et al. Does recall period have an effect on cancer patients' ratings of the severity of multiple symptoms? *J Pain Symptom Manag* 2010;**40**:191–9.
37. Hammer C, Fasching PA, Loehberg CR, et al. Polymorphism in HTR3D shows different risks for acute chemotherapy-induced vomiting after anthracycline chemotherapy. *Pharmacogenomics* 2010;**11**:943–50.