Citation: Clinical and Translational Gastroenterology (2015) 6, e90; doi:10.1038/ctg.2015.19

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Genetic and Non-genetic Factors Associated With Constipation in Cancer Patients Receiving Opioids

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OBJECTIVES: To examine whether the inter-individual variation in constipation among patients receiving opioids for cancer pain is associated with genetic or non-genetic factors.

METHODS: Cancer patients receiving opioids were included from 17 centers in 11 European countries. Intensity of constipation was reported by 1,568 patients on a four-point categorical scale. Non-genetic factors were included as covariates in stratified regression analyses on the association between constipation and 75 single-nucleotide polymorphisms (SNPs) within 15 candidate genes related to opioid- or constipation-signaling pathways (*HTR3E, HTR4, HTR2A, TPH1, ADRA2A, CHRM3, TACR1, CCKAR, KIT, ARRB2, GHRL, ABCB1, COMT, OPRM1,* and *OPRD1*).

RESULTS: The non-genetic factors significantly associated with constipation were type of laxative, mobility and place of care among patients receiving laxatives ($N = 806$), in addition to Karnofsky performance status and presence of metastases among patients not receiving laxatives ($N = 762$) ($P < 0.01$). Age, gender, body mass index, cancer diagnosis, time on opioids, opioid dose, and type of opioid did not contribute to the inter-individual differences in constipation. Five SNPs, rs1800532 in *TPH1*, rs1799971 in *OPRM1*, rs4437575 in *ABCB1*, rs10802789 in *CHRM3*, and rs2020917 in *COMT* were associated with constipation ($P < 0.01$). Only rs2020917 in *COMT* passed the Benjamini–Hochberg criterion for a 10% false discovery rate.

CONCLUSIONS: Type of laxative, mobility, hospitalization, Karnofsky performance status, presence of metastases, and five SNPs within *TPH1*, *OPRM1*, *ABCB1*, *CHRM3*, and *COMT* may contribute to the variability in constipation among cancer patients treated with opioids. Knowledge of these factors may help to develop new therapies and to identify patients needing a more individualized approach to treatment.

Clinical and Translational Gastroenterology (2015) **6,** e90; doi:10.1038/ctg.2015.19; published online 18 June 2015
Subject Category: Functional GI Disorders

INTRODUCTION

The inter-individual variation in analgesic response to opioids is well known. There is also a large inter-individual variability in constipation among both healthy volunteers[1] and cancer patients receiving opioids.[2] Constipation is a significant symptom among cancer patients receiving opioids, with prevalence rates ranging from 50 to 100% and with a potential to significantly impair the quality of life.[3–5] There is substantial evidence suggesting that treatment of constipation in this population can and should be improved. Still, constipation remains poorly recognized and undertreated.[6] Although laxatives are commonly prescribed, there is a surprising lack of evidence to guide the choice of treatment for the individual patient.[7]

Constipation results from a lack of coordination between motility, mucosal transport, and defecation reflexes.[3,8] In normal bowel function, these mechanisms are finely adjusted via the enteric nervous system and a variety of gastrointestinal hormones constituting an intricate interplay between agonists, antagonists and receptors.[3,8] Based on available information about function, physiology, and bowel dysfunction, genetic variants within genes encoding serotonin receptors and associated proteins (*HTR3E*,[9–12] *HTR4*,[13] *HTR2A*,[14,15] and *TPH1*[16]), α_2 adrenergic receptors (*ADRA2A*[14,17]), cholinergic receptors (*CHRM3*[18]), substance P receptor (*TACR1*[14,19,20]), cholecystokinin receptors (*CCKAR*[18,21–23]), the ghrelin-obestatin preproprotein (*GHRL*[24]), and the proto-oncogene c-kit (*KIT*[25,26]) are candidates to influence the presence and intensity of constipation in cancer patients.

Administration of opioids influences the enteric nervous system signaling, delays gastric emptying and intestinal transit, reduce gastrointestinal motility by suppressing the excitability and neurotransmitter release from enteric musculomotor neurones, and inhibit secretion, leading to opioid-induced constipation.[18] The interplay between opioids and bowel physiology is complex, but it has been shown that opioids and α_2-adrenoceptor agonists have similar effects in the rat small intestine,[17] that opioid agonists affect intestinal motility by modulating cholinergic transmission,[18] inhibit

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Received 15 January 2015; accepted 4 May 2015

release of substance P and block the presynaptic CCK-activated acetylcholine release.[18,19] Tryptophan hydroxylase 1 (TPH1) is known to increase in chronic constipation.[16] Chronic morphine administration increase c-Kit expression in bowel fragments of rats.[26] Selective 5-HT4 receptor agonists,[13] 5-HT2 receptor blockers and grehlin have been shown to improve opioid-induced constipation.[15,24] These observations in studies related to opioids and bowel function emphasize the potential influence of the candidate genes identified from factors involved in bowel function in general.

In addition to the genetic variants related to constipation mechanisms, genetic variants affecting the pharmacokinetic and pharmacodynamic properties of opioids may also lead to inter-individual variations in opioid response.[27] Genetic variations within genes encoding proteins involved in absorption, transport (*ABCB1*, adenosine triphosphate-binding cassette, subfamily B, member 1[28–31]), metabolism (*COMT*, catechol-*O*-methyl transferase[32,33]), elimination, receptor binding, and downstream signaling (*OPRM1/K1/D1* opioid receptors[3,34] and *ARRB2*, β-arrestin[34–37]) may contribute to the inter-individual variations in constipation during opioid treatment.[3,27]

There is a lack of knowledge about the causes of inter-individual differences in constipation during opioid treatment, although the association with cancer diagnosis, factors associated with opioid therapy and putative factors influencing the pathogenesis of constipation have been studied previously.[2,5,38,39] Increasing age and female gender,[4] overweight,[40] lower Karnofsky performance status,[39,41,42] hospitalization,[38] longer time on opioids, higher opioid dose,[5] certain opioid types,[14,43] certain cancer diagnoses,[4] presence of metastases,[38,39] and reduced mobility[42,44] are all among the proposed risk factors. However, most of these factors were found not to be significantly associated with the inter-individual variation in constipation in a clinically relevant sample of cancer patients receiving opioids.[2] Knowledge of factors associated with the variation in constipation may help to individualize treatment and avoid unnecessary patient suffering in the future. The present study aimed to identify possible genetic and non-genetic factors associated with the inter-individual variation in constipation among cancer patients receiving opioids.

METHODS

Patients. The European Pharmacogenetic Opioid Study included 2,294 patients receiving opioids for cancer pain, from 17 centers in 11 countries.[45] Included patients were 18 years or older, had a verified diagnosis of malignant disease, agreed to give a blood sample and had received scheduled opioid treatment corresponding to step III at the WHO analgesic ladder for at least 3 days.[46] Patients who lacked a basic proficiency of the language spoken in the study center were excluded. Because some chemotherapies cause constipation and others cause diarrhea,[2] patients receiving chemotherapy were excluded ($N=353$). For the analyses of genetic association we also excluded non-Caucasians ($N=47$) and Greek patients ($N=5$) to minimize heterogeneity. Samples in which no genomic DNA was available ($N=20$) or where all genotyping failed ($N=2$) and patients not answering the question about constipation ($N=299$) were also excluded. Finally, as all patients receiving step III opioids should have laxatives prescribed according to guidelines, we analyzed those receiving laxatives ($N=806$) and those not receiving laxatives ($N=762$) separately, as we did not know the reason for lack of laxative prescription.

The study was approved by ethical committees at each study center or in each country before initialization and performed according to the rules of the Helsinki-declaration. Written informed consent was obtained from all patients before inclusion.

Patients reported constipation and their need to stay in bed or a chair during the day by answering the European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC-QLQ-C30).[47] The constipation intensity and extent of mobility during the past week were assessed on a four-point verbal rating scale with categories of "not at all, a little, quite a bit and very much". The exact questions were: "Have you been constipated?" for constipation and "Do you need to stay in bed or a chair during the day?" for mobility. Whole blood was drawn for pharmacogenetic analyses.

As prevalence and intensity of constipation might also be influenced by a number of non-genetic factors,[2,4,38–42] these were also registered to be included as covariates in the analyses of genetic association. Health-care providers (physician or nurse) registered age, gender, body mass index (BMI), time since start of opioids (months), opioid dose (total oral morphine equivalent daily dose in mg), cancer diagnosis, presence of metastasis, type of laxatives used during the past 24 h, type of opioid, affiliation to department, and country. In addition, the providers assessed Karnofsky performance status,[48] and cognitive function by the mini-mental state examination (MMSE).[49]

SNP selection, genotyping and quality control. Within 16 candidate genes, 88 putative single-nucleotide polymorphisms (SNPs) were selected based on a combination of associations identified in literature, available information in databases,[50–53] their frequency, functionality and their inter-related distance (Supplementary Table 1 online). For SNP selection the SNP browser version 3.5 (Applied Biosciences, Foster City, CA, USA) was used to ensure that all selected SNPs had an expected allele frequency of 10% or more in Caucasians and that they were compatible with assay rules.

Isolation of genomic DNA from EDTA whole blood was performed at HUNT Biobank, Levanger, Norway by using the Gentra Puregene blood kit (QIAGEN Science, MA, USA). The SNPlex Genotyping Platform, including universal SNPlex System kits and reagents and SNP-specific ligation probes, was used (Applied Biosciences). Genotyping was performed according to the supplier's dry DNA protocol. The GeneMapper Software v4.0 (Applied Biosciences) and manual reading was used to analyze the SNPlex signals. Quality control and data cleaning was performed. Samples with low signals not separable from negative controls and samples in which $<90\%$ of SNPs were genotyped were removed prior to analysis and treated as missing data. SNPs with a callrate $<90\%$ and SNPs with inconsistent clustering on inspection were excluded from analyses.

Statistics. Collection and organization of data was performed by The Pain and Palliation Research Group, Norwegian University of Science and Technology. The statistical software STATA version 11.0 was used for all analyses (StataCorp. 2009 *STATA Statistical Software: Release 11*. College Station, TX, USA: StataCorp LP).

Genotype frequencies, allele frequency and carriage were determined and quality checked. SNPs in which no genotypes were recorded, SNPs where genotypes were not in Hardy–Weinberg equilibrium (X^2-test, $P<0.0005$) and SNPs with an observed minor allele frequency (MAF) $<5\%$ were rejected.

Univariate regressions (ordered logistic and linear) were performed to investigate the possible associations between non-genetic factors and intensity of constipation as reported in EORTC question 16. The factors explored were age, BMI, KPS, time on opioids, opioid dose, gender, type of laxative, mobility (as reported by EORTC question 4), type of opioid, department, metastases, and cancer diagnosis. Age, BMI, KPS, and opioid dose were analyzed both as continuous and as dichotomised variables (age ≤ 60 vs. >60, BMI <25 vs. ≥ 25, KPS ≤ 80 vs. >80, dose ≤ 300 mg vs. >300 mg).[5] All factors significantly ($P<0.05$) associated with constipation in univariate analyses were considered for inclusion as covariates in the stepwise multivariate analysis stratified by country. The identified non-genetic covariates were included in stratified multivariate regression analyses on the association between constipation and SNPs within the candidate genes related to the opioid- or constipation-signaling pathways (*HTR3E, HTR4, HTR2A, TPH1, ADRA2A, CHRM3, TACR1, CCKAR, KIT, ARRB2, GHRL, ABCB1, COMT, OPRM1, OPRK1,* and *OPRD1*). These ordered logistic regression analyses, with constipation as the dependent variable (scored 0 for "Not at all", 1 for "A little", 2 for "Quite a bit" and 3 for "Very much") generated β-slopes. 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.

To mitigate the issue of multiple testing we used a 10% false discovery rate reporting the Benjamini–Hochberg (BH) thresholds, the constipation question of EORTC was pre-specified as the primary outcome and the codominant genetic model was prespecified for the primary analyses (dominant, recessive and additive models were exploratory). *P* values <0.01 were interpreted as suggestive of effects that should be evaluated in future studies.

RESULTS

Patients. The demographic and disease-related characteristics of the 1,568 patients included in this study are shown in Table 1. Patients receiving laxatives were similar to those not receiving laxatives regarding age (mean 63 and 61 years), gender (59 and 49% male), BMI (24 and 23 kg/m^2), KPS (60 and 63), mean MMSE total score,[27] time since diagnosis (31 months), presence of metastases (86 and 80%), cancer diagnoses represented, and type of opioids prescribed. There were more out-patients among those not receiving laxatives (29%), as compared with patients receiving laxatives (13%).

Table 1 Patient demographics

	Laxatives (N = 806)		*No laxatives (N = 762)*	
	Mean	***s.d.***	***Mean***	***s.d.***
Age (years)	63.1	11.9	60.6	12.1
Body mass index (kg/m^2)	23.8	4.6	23.3	4.6
Karnofsky performance status (range 0–100)	60.0	16.2	62.7	16.6
Mini mental state, total score (range 0–30)	26.7	3.5	27.2	3.0
Time since diagnosis (months)	31.1	44.8	30.6	44.2
	N	**%**	**N**	**%**
Gender				
Female	333	41.3	390	51.2
Male	473	58.7	372	48.8
Department				
Palliative care unit/hospice	272	33.7	226	29.7
General oncology ward	424	52.6	278	36.5
Surgical ward	7	0.9	35	4.6
Out-patients	103	12.8	223	29.3
Status of opioid treatment				
Opioid recently initiated/titration	158	19.6	140	18.4
Stable dosing	642	79.7	616	80.8
Metastases				
None	114	14.1	156	20.5
One or more	692	85.9	606	79.5
Cancer diagnosis				
Breast	88	10.9	81	10.6
Female reproductive organs	48	6.0	79	10.4
Gastrointestinal	140	17.4	192	25.2
Hematological	38	4.7	39	5.1
Head and neck	34	4.2	62	8.1
Lung	173	21.5	113	14.8
Prostate	131	16.3	60	7.9
Urological	60	7.4	53	7.0
Other or unknown	128	15.9	114	15.0
Type of opioid				
Morphine	366	45.4	254	33.3
Oxycodone	189	23.4	144	18.9
Fentanyl	174	21.6	277	36.4
Other	77	9.6	87	11.4
Country				
Denmark	10	1.2	18	2.4
Finland	8	1.0	17	2.2
Germany	111	13.8	128	16.8
Iceland	65	8.1	50	6.6
Italy	116	14.4	191	25.1
Lithuania	0	0	41	5.4
Norway	271	33.6	130	17.1
Sweden	29	3.6	78	10.2
Switzerland	64	7.9	20	2.6
United Kingdom	132	16.4	89	11.7
Laxative treatment				
Bulk	376	46.7		
Stimulant	175	21.7		
Combination and/or other	253	31.4		
EORTC 16 Constipation				
Not at all	160	19.9	327	42.9
A little	180	22.3	194	25.5
Quite a bit	233	28.9	153	20.1
Very much	233	28.9	88	11.5

EORTC 16, European Organization for Research and Treatment of Cancer Core Quality of Life Question number 16.

Table 2 Non-genetic factors associated with constipation in univariate analyses

	Receiving laxatives (N = 806)			*No laxatives (N = 762)*		
	β	***95% CI***	**P**	***β***	***95% CI***	**P**
Age (years)						
≤ 60 (0)	*0.003*	*– 0.003 to 0.009*	*0.339*	*0.004*	*– 0.002 to 0.010*	*0.187*
>60 (1)	0.084	– 0.070 to 0.239	0.284	0.115	– 0.033 to 0.264	0.128
BMI (kg/m^2)						
<25 (0)	*– 0.007*	*– 0.024 to 0.010*	*0.402*	*– 0.000*	*– 0.016 to 0.016*	*0.997*
≥25 (1)	0.022	– 0.141 to 0.185	0.794	– 0.066	– 0.228 to 0.096	0.424
KPS (range 0–100)						
≤ 80 (0)	*– 0.001*	*– 0.005 to 0.004*	*0.776*	*– 0.003*	*– 0.008 to 0.001*	*0.171*
>80 (1)	– 0.381	– 0.763 to – 0.000	0.050	– 0.457	– 0.750 to – 0.164	0.002
Time since start opioids	– 0.048	– 0.106 to 0.010	0.108	0.048	– 0.009 to 0.104	0.101
Total daily dose (g)	0.141	– 0.264 to – 0.019	0.024	0.148	– 0.033 to 0.328	0.109
Gender						
Male (0), female (1)	0.104	– 0.050 to 0.258	0.185	– 0.079	– 0.228 to 0.070	0.298
Type of opoid						
Morphine (0 = no, 1 = yes), oxycodone (0 = no, 1 = yes), fentanyl (0 = no, 1 = yes), other (0 = no, 1 = yes)	– 0.042	– 0.277 to 0.193	0.725	– 0.138	– 0.315 to 0.039	0.127
Metastases						
None (0), ≥ one (1)	0.167	– 0.049 to 0.382	0.129	0.259	0.076 to 0.441	0.006
Cancer diagnosis						
Other (0), gastrointestinal or female reproductive organs (1)	– 0.116	– 0.295 to 0.063	0.203	– 0.096	– 0.252 to 0.059	0.225
Laxative treatment						
Bulk (0 = no, 1 = yes), stimulant (0 = no, 1 = yes), combination and/or other (0 = no, 1 = yes)	0.212	0.126 to 0.297	<0.001			
Reduced mobility						
Not at all, a little, quite a bit, very much	0.178	0.100 to 0.256	<0.001	– 0.001	– 0.077 to 0.075	0.979
Department						
Outpatient (0), hospitalized (1)	0.533	0.309 to 0.757	<0.001	– 0.079	– 0.242 to 0.085	0.345

BMI, body mass index; CI, confidence interval; KPS, Karnofsky performance status.
Results of linear regression. The results of ordered logistic regression (not shown) were closely similar. The dependent variable, constipation, was scored as 0 for "Not at all", 1 for "A little", 2 for "Quite a bit", and 3 for "Very much". Note: analyses were stratified by country. Age, BMI, and KPS were investigated both as continuous and as dichotomous variables.

Almost 80% were on stable dosing of opioids in both groups. "Quite a bit" or "very much" constipation was reported by 58% of patients receiving laxatives as compared with 32% among those not receiving laxatives.

Association with non-genetic factors. In the univariate analyses, the results of ordered logistic and linear regressions were consistent. Five of the non-genetic factors were considered as significantly associated with the intensity of constipation (Table 2). These were type of laxative, mobility as measured by EORTC question 4 and whether the patient was an outpatient or admitted to a hospital among patients receiving laxatives (all $P<0.001$). Karnofsky performance status ($P=0.002$) and presence of metastases ($P=0.006$) were associated with intensity of constipation among patients not receiving laxatives. In addition, the covariate "total daily opioid dose (mg)" had a P value of 0.024 in univariate analyses among patients treated with laxatives (Table 2). But as this covariate had coefficients that were not very consistent and reliable, was not a covariate among those not receiving laxatives and was not strongly prognostic when included in multivariate analyses (those underlying Table 3), it was dropped for further analyses (see also Supplementary Table 2). The five significant factors were included as covariates in the multivariate regressions of genetic factors. The distributions of the responses for the EORTC constipation score in relation to the identified non-genetic factors are reported in Table 3.

Genotype distributions. The success rates of genotyping and frequencies of genotypes and alleles are shown in Supplementary Table 1 online. Out of the 88 candidate SNPs, 13 were excluded from analyses because of deviation from the Hardy–Weinberg equilibrium or a low observed MAF (< 5%). These were rs34826744 in *HTR4*, rs13306143 and rs3750625 in *ADRA2A*, rs2237037 in *KIT*, rs16954146, and rs7208257 in *ARRB2*, rs34911341 in *GHRL*, rs1202181 in *ABCB1*, rs7815824 in *OPRK1*, rs1042114, rs204048, rs2234918, and rs204076 in *OPRD1*. The remaining 75 SNPs were further analyzed.

Table 3 Non-genetic factors associated with constipation in multivariate analyses

	Receiving laxatives (N = 806)										*No laxatives (N = 762)*									
	Not at all		*A little*		*Quite a bit*		*Very much*		*Total*		*Not at all*		*A little*		*Quite a bit*		*Very much*		*Total*	
	N	%	N	%	N	%	N	%	N	*β* *95% CI* P	N	%	N	%	N	%	N	%	N	*β* *95% CI* P
KPS (range 0–100)																				0.536
≤80 (0)											299	42	176	25	147	21	88	12	710	0.318–0.906
>80 (1)											28	54	18	35	6	12	0	0	52	0.020
Metastases																				1.599
None (0)											86	55	33	21	22	14	15	10	156	1.139–2.243
≥ one (1)											241	40	161	27	131	22	73	12	606	0.007
Laxative treatment																				
Bulk	88	23	98	26	96	26	94	25	376	0.426										
Stimulant	34	19	42	24	66	38	33	19	175	0.273–0.579										
Combination/other	38	15	39	15	71	28	105	42	253	<0.001										
Reduced mobility																				
Not at all	27	36	23	30	15	20	11	14	76	0.272										
A little	42	25	34	20	39	23	52	31	167	0.134–0.409										
Quite a bit	43	15	69	25	95	34	73	26	280	<0.001										
Very much	48	17	53	19	82	29	97	35	280											
Department										0.906										
Outpatient (0)	34	33	29	28	25	24	15	15	103	0.524–1.287										
Hospitalized (1)	126	18	151	21	208	30	218	31	703	<0.001										

BMI, body mass index; CI, confidence interval; KPS, Karnofsky performance status.
Results of linear regression. The results of ordered logistic regression (not shown) were closely similar. The dependent variable, constipation, was scored as 0 for "Not at all", 1 for "A little", 2 for "Quite a bit", and 3 for "Very much". Because of a few missing values, some counts does not add up to 100%.

Association with genetic factors. The non-genetic risk factors identified as statistically significant in Tables 2 and 3 were included in the multivariate analysis underlying Tables 4 and 5, where significant non-genetic risk factors were combined with genetic risk factors in a multivariable model. As shown in Table 4, the genetic factors associated with constipation among patients receiving laxatives were rs1800532 within *TPH1* in a codominant model, rs1799971 within *OPRM1* in additive and dominant models, as well as rs4437575 within *ABCB1* and rs10802789 within *CHRM3* in a dominant model ($P<0.01$). None of these associations passed the BH criterion for a 10% false discovery rate. As shown in Table 5, the genetic factor associated with constipation among patients not receiving laxatives was rs2020917 within *COMT* in a codominant model. This association passed the BH criterion for a 10% false discovery rate.

More patients reported "quite a bit" or "very much" constipation among those not carrying the C-allele of rs1800532 in *TPH1* (64%) and those not carrying the G-allele of rs1799971 in *OPRM1* (63%). More patients reported "quite a bit" or "very much" constipation among those carrying the G-allele of rs4437575 in *ABCB1* (61%), the T-allele of rs10802789 in *CHRM3* (60%) or the T-allele of rs2020917 in *COMT* (36%).

DISCUSSION

The inter-individual differences in constipation among patients receiving opioids are associated with the type of laxative administered, level of mobility, place of care, Karnofsky performance status, presence of metastases and five polymorphisms within *TPH1*, *OPRM1*, *ABCB1, CHRM3,* and *COMT* ($P<0.01$).

The characteristics of included patients (Table 1) were as expected for cancer patients.[54] We found that 58% of patients receiving laxatives and 32% of patients not receiving laxatives reported "quite a bit" or "very much" constipation. These numbers indicate the large inter-individual variation in constipation among cancer patients receiving opioids, with some patients being constipated despite optimized treatment with laxatives and some not experiencing constipation despite high doses of opioids.[55]

In agreement with other studies we observed that type of laxative,[56,57] hospitalization,[38] reduced mobility,[42,44] Karnofsky performance status,[41,42] and presence of metastases[38] influence whether a cancer patient report to experience constipation when receiving opioids.

The results of our study indicate that polymorphisms within *TPH1* may contribute to the inter-individual variations in constipation. Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in enterochromaffin (EC) cell 5-HT biosynthesis. Following luminal chemical and mechanical signals, the EC-cells release 5-HT, which stimulates 5-HT3 and 5-HT4 receptors on primary afferent neurons, inducing secretomotor and peristaltic reflexes of the intestines.[58] A common *TPH1* proximal promoter variant (rs7130929, –347C>A) has been associated with the diarrheal subtype of irritable bowel syndrome (IBS).[59] Because of the distance to polymorphism rs1800532 (also known as 218A>C, located in intron 7) it is difficult to compare the findings of this study with ours. A study among female, Caucasian IBS patients found no association

Table 4 Genetic factors possibly associated with constipation among patients receiving laxatives ($N = 806$)

Gene	Genotype	*Absolute number of patients*									*Multivariate analysis*				P *value alleles*[a]
SNP	*Allele*	*Not at all*		*A little*		*Quite a bit*		*Very much*		*Total*	*OR*	95% CI	P[b]	*Model*	
		N	%	N	%	N	%	N	%						
TPH1															
rs1800532	AA	26	20	21	16	36	27	48	37	131					
	AC	85	22	93	25	101	27	99	26	378	1.457	1.126–1.885	0.004	Codominant	
	CC	42	16	59	22	87	33	79	30	267					
	C	*127*	*20*	*152*	*24*	*188*	*29*	*178*	*28*	*645*					*0.094*
	Not C	*26*	*20*	*21*	*16*	*36*	*27*	*48*	*37*	*131*					
OPRM1															
rs1799971	AA	84	17	97	20	150	31	152	31	483	0.664	0.500–0.882	0.005	Additive	
	AG	35	22	44	28	40	26	37	24	156	1.523	1.110–2.090	0.009	Dominant	
	GG	2	25	3	38	2	25	1	13	8					
	G	*37*	*23*	*47*	*29*	*42*	*26*	*38*	*23*	*164*					*0.005*
	Not G	*84*	*17*	*97*	*20*	*150*	*31*	*152*	*31*	*483*					
ABCB1															
rs4437575	AA	60	24	60	24	64	26	65	26	249	0.687	0.520–0.908	0.008	Dominant	
	AG	64	17	92	24	117	31	107	28	380					
	GG	31	20	23	15	46	30	53	35	153					
	G	*95*	*18*	*115*	*22*	*163*	*31*	*160*	*30*	*533*					*0.028*
	Not G	*60*	*24*	*60*	*24*	*64*	*26*	*65*	*26*	*249*					
CHRM3															
rs10802789	CC	46	22	52	25	61	30	46	22	205	0.667	0.497–0.896	0.007	Dominant	
	CT	53	16	75	23	102	31	101	31	331					
	TT	25	18	31	23	38	28	42	31	136					
	T	*78*	*17*	*106*	*23*	*140*	*30*	*143*	*31*	*467*					*0.013*
	Not T	*46*	*22*	*52*	*25*	*61*	*30*	*46*	*22*	*205*					
COMT															
rs2020917	CC	55	17	66	20	100	31	103	32	324					
	CT	59	22	60	23	77	29	69	26	265	1.202	0.903–1.601	0.207	Codominant	
	TT	9	15	19	32	14	24	17	29	59					
	T	*68*	*21*	*79*	*24*	*91*	*28*	*86*	*27*	*324*					*0.042*
	Not T	*55*	*17*	*66*	*20*	*100*	*31*	*103*	*32*	*324*					

CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
The odds ratios are from ordered logistic regression with constipation as the dependent variable, scored as 0 for "Not at all", 1 for "A little", 2 for "Quite a bit", and 3 for "Very much". Because of a few missing values, some counts does not add up to 100%.
[a]*P* value of unstratified analyses without the inclusion of covariates.
[b]*P* values of ordered logistic regression in the analyses allowing for covariates and stratified by country.

between the diagnosis and five SNPs, including the rs1800532.[60]

Our findings suggest that polymorphisms within *OPRM1* may be associated with intensity of constipation in cancer patients receiving opioids. The non-synonymous SNP rs1799971 in exon 1 (118A > G, Asp40Asn) has repeatedly demonstrated a functional effect.[61] The effect on analgesia and pain sensitivity is extensively studied, with carriers of the minor 118G allele having a decreased analgesic response to morphine and M6G.[27] Interestingly, only a few of the 118A > G-studies have addressed the association with intensity of constipation and no effect was found.[27] However, in these studies, constipation was only measured as a secondary outcome. In the preclinical setting carriage of the 118G allele is associated with lower levels of mu-opioid receptor mRNA and protein, higher potency and mu-opioid receptor affinity for beta-endorphin and lower potency for exogenous opioids.[61] Clinically, carriage of the 118G allele is associated with higher sensitivity to pain, a need for higher opioid doses to reach analgesic effect and an unchanged or lower risk of opioid-related side effects.[61] In agreement with this, we found that more patients reported "quite a bit" or "very much" constipation among those not carrying the G-allele of 118A > G.

Polymorphisms within the *ABCB1* gene (also known as *MDR1*) may influence intensity of constipation as the product of this gene, P-glycoprotein, is a transporter of many drugs, including opioids. As for *OPRM1*, there are many studies addressing the influence of *ABCB1*-polymorphisms on pain sensitivity and opioid analgesia, but only a few on associations with opioid effects other than analgesia.[27] In a study prospectively recruiting 228 cancer patients receiving morphine, genetic variation in the *ABCB1* gene was associated with drowsiness, confusion, and hallucination.[62] No such association was observed with constipation. The polymorphism rs4437575 investigated in our study is located within the same haploblock as the more known 3435C > T in exon 26 (rs1045642). In the present study more patients reported "quite a bit" or "very much" constipation among those carrying the minor G-allele of rs4437575. This finding is as expected, considering the strong linkage between rs4437575 and rs1045642, where carriage of the minor T-allele in the latter SNP is associated with more opioid-related side effects.[63]

The results also indicated possible associations between SNPs in *CHRM3* and constipation in cancer patients receiving opioids. Cholinergic muscarine receptor 3 (CHRM3) is found

Table 5 Genetic factors possibly associated with constipation among patients not receiving laxatives ($N=762$)

Gene	Genotype	*Absolute number of patients (%)*									*Multivariate analysis*				P *value alleles*[a]
SNP	*Allele*	*Not at all*		*A little*		*Quite a bit*		*Very much*		*Total*	*OR*	95% *CI*	P[b]	*Model*	
		N	%	N	%	N	%	N	%						
TPH1															
rs1800532	AA	40	38	28	27	20	19	17	16	105					
	AC	158	43	91	25	72	20	44	12	365	1.009	0.775–1.315	0.945	Codominant	
	CC	120	44	68	25	59	22	25	9	272					
	C	*278*	*44*	*159*	*25*	*131*	*21*	*69*	*11*	*637*					*0.209*
	Not C	*40*	*38*	*28*	*27*	*20*	*19*	*17*	*16*	*105*					
OPRM1															
rs1799971	AA	207	42	129	26	106	21	56	11	498	1.013	0.758–1.353	0.932	Additive	
	AG	50	41	27	22	31	25	15	12	123	0.960	0.676–1.363	0.820	Dominant	
	GG	6	40	5	33	3	20	1	7	15					
	G	*56*	*41*	*32*	*23*	*34*	*25*	*16*	*12*	*138*					*0.632*
	Not G	*207*	*42*	*129*	*26*	*106*	*21*	*56*	*11*	*498*					
ABCB1															
rs4437575	AA	102	46	48	22	48	22	23	10	221					
	AG	147	39	98	26	81	22	47	13	373	0.867	0.645–1.165	0.345	Dominant	
	GG	71	47	41	27	23	15	16	11	151					
	G	*281*	*54*	*139*	*27*	*104*	*20*	*63*	*12*	*524*					*0.425*
	Not G	*102*	*46*	*48*	*22*	*48*	*22*	*23*	*10*	*221*					
CHRM3															
rs10802789	CC	80	41	50	26	38	19	28	14	196					
	CT	131	46	69	24	54	19	32	11	286	1.119	0.816–1.534	0.484	Dominant	
	TT	46	42	25	23	26	24	12	11	109					
	T	*177*	*45*	*94*	*24*	*80*	*20*	*44*	*11*	*395*					*0.321*
	Not T	*80*	*41*	*50*	*26*	*38*	*19*	*28*	*14*	*196*					
COMT															
rs2020917	CC	143	47	70	23	64	21	28	9	305	0.606	0.454–0.809	**<0.001**	Codominant	
	CT	100	35	78	27	66	23	42	15	286					
	TT	27	55	11	22	9	18	2	4	49					
	T	*127*	*38*	*89*	*27*	*75*	*22*	*44*	*13*	*335*					*0.024*
	Not T	*143*	*47*	*70*	*23*	*64*	*21*	*28*	*9*	*305*					

CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
The odds ratios are from ordered logistic regression with constipation as the dependent variable, scored as 0 for "Not at all", 1 for "A little", 2 for "Quite a bit", and 3 for "Very much". Because of a few missing values, some counts does not add up to 100%.
[a]*P* value of unstratified analyses without the inclusion of covariates
[b]*P* values of ordered logistic regression in the analyses allowing for covariates and stratified by country. Associations in **bold** passed the Benjamini–Hochberg criterion for selection requiring a 10% false discovery rate correction for multiple testing.

in the intestinal wall,[64] and CHRM3 antagonists have been shown to inhibit intestinal motility.[65] Genetic variation within the *CHRM3* gene (rs3738435) has been tested for an association with IBS and specifically for an association with the constipation subtype, but no such associations were found.[66] The SNP rs10802789, also known as c.-249-8806C > T, has been associated with intensity of nausea/vomiting in a previous study.[67] To our knowledge, the exact functional consequence of this polymorphism is still unknown.

The association between constipation and rs2020917 in *COMT* among cancer patients not receiving laxatives passed the BH criterion. More patients reported "quite a bit" or "very much" constipation among those carrying the T-allele of rs2020917 in *COMT* (36%). The variant rs2020917 is located in the 5′ regulatory promoter of the membrane-bound-COMT isoform and it has been shown to alter nuclear protein binding patterns, thereby upregulating transcription and possibly increasing COMT enzyme activity.[68] On the contrary, it has also been demonstrated that the haploblock containing the T-allele of rs2020917 and the C-allele of the nearby rs737865 is associated with reduced *COMT*-transcription.[69] Decreased enzyme-activity, as coded by the Met-allele of the Val158Met (rs4680) variant has been associated with enhanced activation of dopaminergic neurotransmission and lower opioid-dose requirement.[70] In animal models, chronic activation of dopaminergic neurotransmission reduces the neuronal content of enkephalin peptides,[71] leading to an upregulation of mu-opioid receptors.[72] Taken together, our finding agrees with the existing literature on lower opioid-dose requirements and possibly increased adverse effects associated with reduced *COMT*-transcription and enzyme-activity.

There are several challenges of candidate gene association research, and we recognize some in the present study. First, there is a lack of a stringent definition of constipation among cancer patients receiving opioids. Hence, comparison of results between studies is difficult and there is no agreement on definition of the phenotype.[73] This study, including more patients than other studies addressing genetic variability related to opioid effects, utilized the EORTC QLQ-C30, a well-validated assessment tool, formally translated into many languages to define the phenotype. Other studies may also include objective measures such as number of stools and similar outcomes. Second, symptom intensity was registered for the past week, whereas administration of laxatives was registered for the past 24 h. However, we believe use of laxatives was related to symptom intensity as assessments for the past 24 h and the past week are closely related in cancer patients.[74] Third, this study did not take into consideration gene–gene interactions, gene–environment interactions or epigenetics. However, genetic features in favor of the present study are that genes and polymorphisms were chosen based on known biology and pathophysiology, population stratification was avoided by only including Caucasians, measures have been undertaken to control for false positive findings, more than a few candidate SNPs were included in the analyses, and potential clinical confounding factors were identified and included in the analyses. Finally, as no replication sample was included, the findings should be repeated in an independent study before the associations could be regarded as conclusive.

CONCLUSION

This study suggests that type of laxative, mobility, hospitalization, Karnofsky performance status, presence of metastases and five SNPs within *TPH1*, *OPRM1*, *ABCB1*, *CHRM3,* and *COMT* are associated with the variability in constipation among cancer patients treated with opioids ($P<0.01$). Only rs2020917 in *COMT* passed the BH criterion for a 10% false discovery rate. Genetic associations can be helpful to elucidate the relevant biological mechanisms for constipation in patients treated with opioids. These biological mechanisms can therefore be identified as targets for developing new and improved therapy for constipation in patients receiving opioids. Before introduction of genetic testing in routine patient care, large prospective studies are needed to determine whether genetic testing of polymorphisms helps to predict the risk and treatment of constipation among cancer patients receiving opioids, and whether this is a cost-effective approach.

CONFLICT OF INTEREST

Guarantor of the article: Eivor A. Laugsand, MD, PhD.
Specific author contributions: All authors contributed to the conception and design of the study, interpreted the data, revised the manuscript critically for important intellectual content and approved the final version. E.A.L. drafted the manuscript.
Financial 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.
Potential competing interests: None.

Acknowledgments. We are grateful to all the researchers involved in The European Pharmacogenetic Opioid Study (EPOS) and to Gunnhild Jakobsen who organized the collection of data.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ There is inter-individual variation in both analgesic response and constipation among patients receiving opioids.
- ✓ There is a surprising lack of evidence to guide the choice of laxative treatment for the individual patient.

WHAT IS NEW HERE

- ✓ Type of laxative, mobility, hospitalization, Karnofsky performance status, presence of metastases and five SNPs within *TPH1*, *OPRM1*, *ABCB1*, *CHRM3*, and *COMT* may contribute to the variability in constipation among cancer patients receiving opioids.
- ✓ Our findings reveal relevant biological mechanisms for constipation that might contribute to developing new and improved therapy for constipation in patients receiving opioids.

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