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.

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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 and cancer patients receiving opioids. 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. There is substantial evidence suggesting that treatment of constipation in this population can and should be improved. Still, constipation remains poorly recognized and undertreated. Although laxatives are commonly prescribed, there is a surprising lack of evidence to guide the choice of treatment for the individual patient.

Constipation results from a lack of coordination between motility, mucosal transport, and defecation reflexes. 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. Based on available information about function, physiology, and bowel dysfunction, genetic variants within genes encoding serotonin receptors and associated proteins (HTR3E, HTR4, HTR2A, TPH1, ADRA2A, CHRM3, TACR1, CCKAR, KIT, ARRB2, GHRL, ABCB1, COMT, OPRM1, and OPRD1).

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release of substance P and block the presynaptic CCK-activated acetylcholine release.\textsuperscript{18,19} Tryptophan hydroxylase 1 (TPH1) is known to increase in chronic constipation.\textsuperscript{16} Chronic morphine administration increases K+ expression in bowel fragments of rats.\textsuperscript{26} Selective 5-HT4 receptor agonists,\textsuperscript{13} 5-HT2 receptor blockers and ghrelin have been shown to improve opioid-induced constipation.\textsuperscript{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.\textsuperscript{22} Genetic variations within genes encoding proteins involved in absorption, transport (\textit{ABCB1}, adenosine triphosphate-binding cassette, subfamily B, member 128), metabolism (\textit{COMT}, catechol-O-methyl transferase),\textsuperscript{32,33} elimination, receptor binding, and downstream signaling (\textit{OPRM1/K1/D1} opioid receptors\textsuperscript{3,34} and \textit{ARRB2}, \(\beta\)-arrestin)\textsuperscript{34–37} may contribute to the inter-individual variations in constipation during opioid treatment.\textsuperscript{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.\textsuperscript{2,5,38,39} Increasing age and female gender,\textsuperscript{\textit{a}} overweight,\textsuperscript{40} lower Karnofsky performance status,\textsuperscript{39,41,42} hospitalization,\textsuperscript{38} longer time on opioids, higher opioid dose,\textsuperscript{5} certain opioid types,\textsuperscript{14,44} certain cancer diagnoses,\textsuperscript{4} presence of metastases,\textsuperscript{38,39} and reduced mobility\textsuperscript{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.\textsuperscript{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.

\textbf{METHODS}

\textbf{Patients.} The European Pharmacogenetic Opioid Study included 2,294 patients receiving opioids for cancer pain, from 17 centers in 11 countries.\textsuperscript{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.\textsuperscript{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,\textsuperscript{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).\textsuperscript{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,\textsuperscript{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,\textsuperscript{48} and cognitive function by the mini-mental state examination (MMSE).\textsuperscript{49}

\textbf{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,\textsuperscript{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.
analyses, with constipation as the dependent variable (scored ≥ observed minor allele frequency (MAF) as dichotomised variables (age KPS, and opioid dose were analyzed both as continuous and department, metastases, and cancer diagnosis. Age, BMI, mobility (as reported by EORTC question 4), type of opioid, KPS, time on opioids, opioid dose, gender, type of laxative, EORTC question 16. The factors explored were age, BMI, non-genetic factors and intensity of constipation as reported in performed to investigate the possible associations between constipation and SNPs within the candidate genes (related to the opioid- or constipation-signaling pathways stratified multivariate regression analyses on the association between constipation and SNPs within the candidate genes evaluated in future studies.

RESULTS

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/m²) | 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   | 44.4  |

| Gender                | N      | %     | N      | %     |
|-----------------------|--------|-------|--------|-------|
| Female                | 333    | 41.3  | 390    | 51.2  |
| Male                  | 473    | 58.7  | 372    | 48.8  |

| Department            | N      | %     | N      | %     |
|-----------------------|--------|-------|--------|-------|
| 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 | N      | %     | N      | %     |
|----------------------------|--------|-------|--------|-------|
| Opioid recently initiated/titration | 158    | 19.6  | 140    | 18.4  |
| Stable dosing             | 642    | 79.7  | 616    | 80.8  |

| Metastases              | N      | %     | N      | %     |
|-------------------------|--------|-------|--------|-------|
| None                    | 114    | 14.1  | 156    | 20.5  |
| One or more             | 692    | 85.9  | 606    | 79.5  |

| Cancer diagnosis         | N      | %     | N      | %     |
|--------------------------|--------|-------|--------|-------|
| Breast                   | 88     | 10.9  | 81     | 10.6  |
| Female reproductive organs | 48     | 6.0   | 79     | 10.4  |
| Gastrointestinal         | 140    | 17.4  | 152    | 20.2  |
| Hematomatological        | 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           | N      | %     | N      | %     |
|--------------------------|--------|-------|--------|-------|
| 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                  | N      | %     | N      | %     |
|--------------------------|--------|-------|--------|-------|
| 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       | N      | %     | N      | %     |
|--------------------------|--------|-------|--------|-------|
| 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.
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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/m²)                    |                  |            |         |                  |            |         |
| < 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       |                  |            |         |                  |            |         |
| Total daily dose (g)           | -0.048           | -0.106 to 0.010 | 0.108    | 0.048            | -0.009 to 0.104 | 0.101    |
| 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 opioid                 |                  |            |         |                  |            |         |
| 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, rs284048, rs2234918, and rs204076 in OPRD1. The remaining 75 SNPs were further analyzed.
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 and rs1799971 within TPH1 in a codominant model, rs10802789 within OPRM1 in additive and dominant models, and rs4437575 within ABCB1 in a codominant model. This association 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 in a dominant model (P<0.001). None of these associations were included in the multivariate analysis underlying Tables 4 and 5, where significant non-genetic risk factors were identified as statistically significant in Tables 2 and 3 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,58 reduced mobility,52,44 Karnofsky performance status,51,42 and presence of metastases59 influence whether a cancer patient report to experience constipation when receiving opioids. The results of our study indicate that 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,58 reduced mobility,52,44 Karnofsky performance status,51,42 and presence of metastases59 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 hydroxylation (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
constipation and no effect was found. However, in these studies have addressed the association with intensity of morphine and M6G. Interestingly, only a few of the 118A minor 118G allele having a decreased analgesic response to and pain sensitivity is extensively studied, with carriers of the beta-endorphin and lower potency for exogenous opioids. 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. 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. 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.

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

| Gene  | Genotype | Absolute number of patients |  |  |  |  |  |
|-------|----------|-----------------------------|---|---|---|---|---|
| SNP   | Allele   | Not at all | A little | Quite a bit | Very much | Total | OR  | 95% CI | P  | Model |
|       |          | N | % | N | % | N | % | N | % |      |       |
| TPH1  | rs1800532 | AA | 26 | 20 | 21 | 16 | 36 | 27 | 48 | 37 | 131 | 1.457 1.126–1.885 0.004 Codominant |
|       |          | AC | 85 | 22 | 93 | 25 | 101 | 27 | 99 | 26 | 378 |                   |
|       |          | 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 | 32 | 16 | 38 | 28 | 42 | 31 | 136 |                   |
|       |          | 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 | 18 | 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 | 1.202 0.903–1.601 0.207 Dominant |
|       |          | CT | 59 | 22 | 60 | 23 | 77 | 29 | 69 | 26 | 265 |                   |
|       |          | 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%.

P value of unstratified analyses without the inclusion of covariates.

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.

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. 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. Interestingly, only a few of the 118A-G-studies have addressed the association with intensity of constipation and no effect was found. 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. 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. 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. In a study prospectively recruiting 228 cancer patients receiving morphine, genetic variation in the ABCB1 gene was associated with drowsiness, confusion, and hallucination. 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.
Table 5  Genetic factors possibly associated with constipation among patients not receiving laxatives (N=762)

| Gene | SNP | Genotype | Absolute number of patients (%) | Multivariate analysis | P value alleles<sup>a</sup> |
|------|-----|----------|---------------------------------|----------------------|---------------------------|
|      |     |          | Not at all | A little | Quite a bit | Very much | Total | OR  | 95% CI | P<sup>b</sup> | Model |
|      |     | Allele   | N  %       | 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 |      |        |           |       |
|      |       | Not C    | 40 38 | 28 27 | 20 19 | 17 16 | 105 |      |        |           | 0.209 |
| OPRM1 | rs179971 | 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 | 0.867 | 0.645–1.165 | 0.345 | Dominant |
|      |       | AG       | 147 39 | 98 26 | 81 22 | 47 13 | 373 |      |        |           |       |
|      |       | 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 | 1.119 | 0.816–1.534 | 0.484 | Dominant |
|      |       | CT       | 131 46 | 69 24 | 54 19 | 32 11 | 286 |      |        |           |       |
|      |       | 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%.

<sup>a</sup>P value of unstratified analyses without the inclusion of covariates

<sup>b</sup>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.
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. 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. 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. In animal models, chronic activation of dopaminergic neurotransmission reduces the neuronal content of enkephalin peptides, leading to an upregulation of mu-opioid receptors. 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. 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. 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.

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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.
1. Sykes NP, A volunteer model for the comparison of laxatives in opioid-related constipation. J Pain Symptom Manage 2001; 19: 366–369.
2. Drenney J, Ross J, Grettom S et al. Constipation in cancer patients on morphine. Support Care Cancer 2006; 14: 453–459.
3. Kurt A, Sessler D. Opioid-induced bowel dysfunction: pathophysiology and potential new therapies. Drugs 2003; 63: 649–671.
4. Manclini IL, Hantson J, Neumann CM et al. Opioid type and other clinical predictors of laxative dose in advanced cancer patients: a retrospective study. J Pain Palliat Care Pharmacother 2006; 20: 3–49.
5. Clark K, Smith JM, Cunow DC. The prevalence of bowel problems reported in a palliative care population. Drugs 2002; 63: 649–671.
6. Laugsand EA, Jakobsen C, Kaasa S et al. Inadequate symptom control in advanced cancer patients across Europe. Support Care Cancer 2011; 19: 2005–2014.
7. Miles CL, Fellowes D, Goodman ML et al. Laxatives for the management of constipation in palliative care patients. Cochrane Database Syst Rev 2006;CD003448.
8. Mantey J, Bruera E. Constipation in advanced cancer patients. Support Care Cancer 1999; 7: 356–364.
9. Sanger GJ, 5-hydroxytryptamine and the gastrointestinal tract: where next? Trends Pharmacol Sci 2008; 29: 465–471.
10. Niessler B, Frank B, Kaperle J et al. Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. Gene 2003; 310: 101–111.
11. Wouters MM, Faruqui G, Schennach M. 5-HT receptors on intestinal cells of Cajal, smooth muscle and enteric nerves. Neurogastroenterol Motil 2007; 19: 5–12.
12. Kaperle J, Houghton LA, Moennikes H et al. First evidence for an association of a functional variant in the microRNA-150 target site of the serotonin receptor-type 3E gene with diarrhea predominant irritable bowel syndrome. Hum Mol Genet 2008; 17: 2967–2977.
13. Moulin DE, Rykx A, Kerstens R et al. Randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety of prucalopride (Resolor®) in patients with opioid-induced constipation. Gastroenterology 2008; 134: A82–A86.
14. DeLuca A, Coupar IM. Insights into opioid action in the intestinal tract. Pharmacol Therapeut 1996; 69: 103–115.
15. Dhasmana KM, Banerjee AK, Faithfull NS et al. Role of 5-hydroxytryptamine receptors to narcotic-induced reduction in gastrointestinal transit in rats. Zhongguo Yao Li Xue Bao 1996; 28: 499–503.
16. Costedo MM, Coates MD, Brooks EM et al. Mucosal serotonin signaling is altered in chronic constipation but not in opioid-induced constipation. Am J Gastroenterol 2010; 105: 1173–1180.
17. Hikasa Y, Akiba T, Iino Y et al. Central alpha-adrenoceptor subtypes involved in the emetic response to morphine. Eur J Pharmacol 1992; 229: 241–251.
18. Wood JD, Galligan JJ. Functions of opioid in the enteric nervous system. Neurogastroenterol Motil 2004; 16 (Suppl 2): 17–28.
19. Garzon J, Holt V, Herz A. Cholecystokinin octapeptide activates an opioid mechanism in the guinea-pig ileum: a possible role for substance P. Eur J Pharmacol 1997; 336: 361–370.
20. Garcia-Barcelo M, King SK, Miao XP et al. Application of HapMap data to the evaluation of candidate genes for pediatric slow transit constipation. J Pediatr Surg 2007; 42: 666–671.
21. Wiesenfeld-Hallin Z, de Araujo L, Gasser A et al. Cholecystokinin/oipdoid interactions. Gastroenterology 2008; 134: A82–A86.
22. Poirats P, Polvinio WJ, Rocheleau B. Gastrointestinal effect of ghrelin analog RC-113 in the rat: effect on post-operative and on morphine induced ileus. Peptides 2005; 26: 1598–1601.
23. Baranch T, Zurwalski D, Gil K et al. Peripheral mechanisms of intestinal dysmotility in the morphine tolerant and dependent rats. J Pharmacol Exp Ther 1999; 291: 79–85.
24. Poirats P, Polvinio WJ, Rocheleau B. Gastrointestinal effect of ghrelin analog RC-113 in the rat: effect on post-operative and on morphine induced ileus. Peptides 2005; 26: 1598–1601.
25. Tong WD, Liu BH, Zhang LY et al. Analysis of the c-kit gene in patients with slow transit constipation. Gut 2005; 56: 1207–1208.
26. Manclini IL, Hanson J, Neumann CM et al. First evidence for an association of a functional variant in the microRNA-150 target site of the serotonin receptor-type 3E gene with diarrhea predominant irritable bowel syndrome. Hum Mol Genet 2008; 17: 2967–2977.
27. Muslin AL, Rykx A, Kerstens R et al. Randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety of prucalopride (Resolor®) in patients with opioid-induced constipation. Gastroenterology 2008; 134: A82–A86.
28. DeLuca A, Coupar IM. Insights into opioid action in the intestinal tract. Pharmacol Therapeut 1996; 69: 103–115.
29. Dhasmana KM, Banerjee AK, Faithfull NS et al. Role of 5-hydroxytryptamine receptors to narcotic-induced reduction in gastrointestinal transit in rats. Zhongguo Yao Li Xue Bao 1996; 28: 499–503.
30. Costedo MM, Coates MD, Brooks EM et al. Mucosal serotonin signaling is altered in chronic constipation but not in opioid-induced constipation. Am J Gastroenterol 2010; 105: 1173–1180.
31. Hikasa Y, Akiba T, Iino Y et al. Central alpha-adrenoceptor subtypes involved in the emetic response to morphine. Eur J Pharmacol 1992; 229: 241–251.
32. Wood JD, Galligan JJ. Functions of opioid in the enteric nervous system. Neurogastroenterol Motil 2004; 16 (Suppl 2): 17–28.
33. Garzon J, Holt V, Herz A. Cholecystokinin octapeptide activates an opioid mechanism in the guinea-pig ileum: a possible role for substance P. Eur J Pharmacol 1997; 336: 361–370.
34. Garcia-Barcelo M, King SK, Miao XP et al. Application of HapMap data to the evaluation of candidate genes for pediatric slow transit constipation. J Pediatr Surg 2007; 42: 666–671.
35. Wiesenfeld-Hallin Z, de Araujo L, Gasser A et al. Cholecystokinin/oipdoid interactions. Gastroenterology 2008; 134: A82–A86.
36. Poirats P, Polvinio WJ, Rocheleau B. Gastrointestinal effect of ghrelin analog RC-113 in the rat: effect on post-operative and on morphine induced ileus. Peptides 2005; 26: 1598–1601.
37. Tong WD, Liu BH, Zhang LY et al. Analysis of the c-kit gene in patients with slow transit constipation. Gut 2005; 56: 1207–1208.
38. Baranch T, Zurwalski D, Gil K et al. Peripheral mechanisms of intestinal dysmotility in the morphine tolerant and dependent rats. J Pharmacol Exp Ther 1999; 291: 79–85.
39. Skorpen F, Laugsand EA, Kleopstad P et al. Variable response to opioid treatment: any genetic predictors within sight? Palliat Med 2006; 22: 310–327.
40. Campa D, Gioia A, Tomei A et al. Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief. Clin Pharmacol Ther 2008; 83: 559–566.
41. Coulbault L, Beausier M, Verslype C et al. Environmental and genetic factors associated with morphine response in the postoperative period. Clin Pharmacol Ther 2006; 79: 316–324.
42. Fujita K-I, Ando Y, Yamamoto W et al. Association of UGT2B7 and ABCB1 genotypes with morphine-induced adverse drug reactions in Japanese patients with cancer pain. Cancer Chemother Pharmacol 2010; 65: 251–258.
43. Oostenbrug LE, Dijkstra G, Nolte IM et al. Absence of association between the multidrug resistance (MDR1) gene and inflammatory bowel disease. Scand J Gastroenterol 2006; 41: 114–118.
44. Raekvist TT, Kleopstad P, Baar C et al. The Val188Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients. Pain 2005; 116: 73–78.
45. Obayashi K, Olsson M, Anani I et al. Impact of serotonin transporter and catechol-O-methyl transferase genes polymorphism on gastrointestinal dysfunction in Swedish and Japanese familial amyloidoic polyneuropathy patients. Clin Chim Acta 2008; 398: 1–14.
70. Rakvag 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.

71. Steiner H, Gerfen CR. Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. Exp Brain Res 1998; 123: 60–76.

72. Chen JF, Aloyo VJ, Weiss B. Continuous treatment with the D2 dopamine receptor agonist quinpirole decreases D2 dopamine receptors, D2 dopamine receptor messenger RNA and proenkephalin messenger RNA, and increases mu opioid receptors in mouse striatum. Neuroscience 1993; 54: 669–680.

73. Gaertner J, Siemens W, Camilleri M et al. Definitions and outcome measures of clinical trials regarding opioid-induced constipation: a systematic review. J Clin Gastroenterol 2015; 49: 9–16.

74. 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: 191–199.

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