Original Article

Genomic Study in Opioid-Treated Cancer Patients Identifies Variants Associated With Nausea-Vomiting

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Abstract

Context. Opioids are the mainstay therapy for patients affected by cancer pain. However, about 10%–20% of patients do not benefit from the received analgesic treatment or experience side effects. Genetic variability might account for the variation in individual responses to opioids, both in terms of efficacy and toxicity.

Objectives. The aim of this genome-wide association study (GWAS) was to identify genetic markers of opioid toxicity, in terms of nausea-vomiting.

Methods. Cancer patients receiving morphine, oxycodone, buprenorphine, and fentanyl were recruited from different European countries. Data about toxicity (nausea-vomiting score, NVS) and other relevant clinical information were collected, as well as genotyping data. Regression analysis between genotypes of 2052 patients and NVS was performed, using appropriate covariates, with REGENIE software.

Results. We found 65 variants associated with NVS (*P*-value $< 1.0\times10^{-5}$). Of note, 14 intronic variants on chromosome 2 were in *NPAS2* gene, encoding a circadian transcription factor reported to play a role in another opioid side effect, the alteration of sleep. Some of these variants were previously identified as splicing quantitative trait loci of the *NPAS2* gene.

Conclusions. This is the first GWAS, performed in more than two thousand individually genotyped patients treated with opioids for cancer pain, that investigated the genetic bases of opioid-induced nausea-vomiting. Although further studies are needed to confirm our findings and to characterize the functional role of the identified variants, our results emphasize the importance of performing large pharmacogenomic studies to identify germline variants associated with opioid response, with the ultimate goal of tailoring cancer pain therapies. J Pain Symptom Manage 2025;69:175–182.

Key Words

Pharmacogenomics, GWAS, cancer pain, side-effects, morphine, fentanyl

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Accepted for publication: 30 October 2024.

0885-3924/$ - see front matter
https://doi.org/10.1016/j.jpainsymman.2024.10.033

Key Message

This European pharmacogenomic study of the response to opioids, in more than two thousand advanced cancer patients, identifies germline variants associated with nausea-vomiting side effect. The identified polymorphisms map in and might affect the splicing of *NPAS2* gene, encoding a circadian transcription factor already involved in sleep disruption by opioids.

Introduction

Advanced cancer patients experiencing moderate to severe pain are usually treated with opioids according to the third step of the World Health Organization (WHO) analgesic ladder (i.e., morphine, oxycodone, fentanyl, and buprenorphine) for relieving pain.[1] Not only is opioid therapy for cancer-related pain noneffective in some patients, but it also frequently causes side effects, including nausea and vomiting.[2,3] Indeed, nausea is experienced by half of cancer patients receiving opioids (range 3%−85%), whereas vomiting occurs less frequently (4%−50%).[4]

We and others previously hypothesized that genetic factors are involved in individual susceptibility to nausea and vomiting side effects. Indeed, a twin study demonstrated that 59% of phenotypic variability in alfentanil-induced nausea was explained by genetics.[5] Some candidate-gene studies investigated polymorphisms in genes involved in opioid pathways or metabolism and reported associations with genetic variants and the interindividual variability in experiencing nausea and vomiting in cancer patients receiving opioids. For instance, a study on 16 candidate genes in 1579 European patients reported significant associations among clinical characteristics, variants in the *HTR3B*, *COMT* and *CHRM3* genes and nausea and vomiting in cancer patients treated with opioids.[6] Additionally, a Japanese study in 32 cancer patients revealed an association between the low frequency of nausea and the UGT2B7*2 genotype.[7]

We previously carried out an exome-wide association study[8] to overcome another limitation of the candidate-gene approach, which analyzes only a few genes, and does not take into account the genetic heterogeneity underlying a complex phenotype such as nausea and vomiting related to opioid therapy. However, our study was carried out on DNA pools and only one single nucleotide polymorphism (SNP) associated with nausea/vomiting in the discovery series was confirmed in the validation series, but with the opposite effect on the phenotype.

Herein, we individually genotyped by SNP-array more than two thousand European cancer patients treated with opioids for pain, and we carried out the largest genome-wide association study on individual susceptibility to nausea and vomiting to date.

Methods

Patients Series, Data Collection, and Materials

This study included 2193 European adult patients with locally advanced or metastatic tumors. These patients received step III WHO opioids to treat cancer pain. They were part of three studies: CERP, an Italian multicenter, randomized, and longitudinal phase IV clinical trial[9]; EPOS, a European multicentric and cross-sectional study[10]; and MOLO: an Italian, longitudinal study.[11] The inclusion criteria for the three studies are listed in Supplement Table 1.

The study protocol was approved by the Committees for Ethics of each recruiting hospital contributing to the EPOS and CERP studies and by the Ethics Committee of the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan (Italy), for the MOLO study (INT 153/13), and the genetic study the genetic study (INT 20/20). The research was conducted in accordance with the tenets of the Declaration of Helsinki. Patients signed a written informed consent form to agree to the use of their biological samples and data for the purposes of opioid pharmacogenomic research. Personal and clinical information was collected, such as age, sex, country of origin, cancer diagnosis, chemotherapy treatments at the time of recruitment, opioid drug and dose (morphine milligram equivalents, MME were calculated),[12] the response to opioids and their quality of life, such as incidence and type of side effects (including nausea and vomiting). In the two longitudinal studies (MOLO and CERP) data were collected at the enrollment and in five following visits (72 hours, 7, 14, 21 and 28 days after recruitment and while receiving opioid antalgic therapy), while for EPOS data were collected at a unique time point during opioid treatment. Specifically, nausea and vomiting were assessed using patient-reported outcome measures (the European Organization for Research and Treatment of Cancer's Core Quality of Life Questionnaire, EORTC QLQ-C30, for EPOS patients and the Therapy Impact Questionnaire, TIQ, for CERP and MOLO patients) consisting of four-point rating scales that were converted in numerical values from one to four. We calculated a composite nausea vomiting score (NVS), as already described in,[8] following the standardized procedure reported in the EORTC QLQ-C30 Scoring Manual[13] to calculate the mean of the scores for nausea and vomiting, and finally to linearly transform this raw score into the composite NVS, ranging from 0 to 100.

For each retrospectively recruited patient from EPOS and CERP, genomic DNA samples were already available at Fondazione IRCCS Istituto Nazionale dei Tumori. Peripheral blood samples from prospectively enrolled patients were collected. Genomic DNA was extracted, using the DNeasy Blood & Tissue kit

(Qiagen), and fluorometrically quantified using the Quant-iT PicoGreen dsDNA Assay (Thermo Fisher Scientific).

Multivariable Linear Regression With Clinical Variables

To understand which clinical variables were significantly associated with our phenotype of interest (NVS), a multivariable linear regression was performed using the glm() function (generalized linear model) in the R environment. The clinical variables in the model were sex, age, genotyping batch, the study in which each patient was recruited, the administered opioid, morphine-equivalent dose, tumor diagnosis, and chemotherapy treatment. A stepwise model selection based on Akaike information criterion (AIC) was performed and the selected variables were then used as covariates in the GWAS.

Genotyping

Genome-wide genotyping was carried out using Axiom Precision Medicine Research Arrays on a GeneTitan multichannel instrument (Thermo Fisher Scientific). We performed the genotype variant calling using the "Best Practice Workflow" and default quality check (QC) settings (except for the average call rate for passing samples ≥ 97%) with Axiom Analysis Suite v.5.01.38. After removing samples that failed Axiom QC, we extracted the genotypes of our patients and converted them into binary PLINK format. Per-sample and per-marker QC was carried out using the PLINK software v1.921[14]: in detail, we filtered out samples with discrepancies between the collected sex and that imputed from the genotypes, samples with missing call rate greater than 5%, and duplicated or related individuals. Variants in high Hardy-Weinberg (HW) disequilibrium (P-value < 1.0×10^{-6}), with missing genotype rate exceeding 1%, and minor allele frequency (MAF) < 1% were removed. We also filtered out polymorphisms mapping in regions with extended linkage disequilibrium (LD)[15]; finally, we retained only biallelic and autosomal variants.

A principal component analysis (PCA) was performed with PLINK v.2[16] to correct for population stratification. We projected the first four principal components (PCs) of our patients together with those of 1563 individuals from five different populations, selected from 1000 Genomes Project[17]: Africans, Americans, South-East Asians, East Asians, and Europeans. The scatterplot of the PC1 *vs.* PC2 and PC3 *vs.* PC4 was plotted by grouping and coloring dots at a continental level. Patients who did not cluster with Europeans were removed from the dataset.

Genotype imputation to the whole genome sequence was performed using the TopMED Imputation Server, setting GRCh38/hg38 as build, TopMED as reference Panel, and phasing the data with Eagle v2.4.[18–21] The imputed genotypes were then filtered to exclude rare variants (MAF < 2%) and those with a low-quality imputation (R^2 info score ≤ 0.3).[22]

Genome-Wide Association Study

We performed a Genome-Wide Association Study (GWAS) using the REGENIE software,[23] using default settings of the pipeline (https://rgcgithub.github.io/regenie/options/), that consisted of two different steps. In step 1, ridge regression was performed, using the nonimputed dataset, to define genetic predictors, calculated with the Leave One Chromosome Out method, to be used in the second step. Then, Step 2 of REGENIE analysis was tested on 7,669,761 imputed germline variants. The regression model in the REGENIE pipeline was performed with the following covariates: age, sex, the study in which each patient was recruited (coded as a dummy variable), and the opioid morphine-equivalent dose. To graphically visualize the associations with NVS, a Manhattan plot was drawn using the qqman library,[24] and the function manhattan () in R environment. Intronic variants of the *NPAS2* gene were searched in the GTEx portal (accessed on 08/24/2023) for a possible role as splicing QTL.

Results

The whole patient series recruited in the study comprised 2193 patients. Some samples were excluded from the analysis because they failed quality check steps (Supplement Fig. 1A): in detail, 30 samples failed the Axiom Quality Controls pipeline, 23 samples were removed due to sex inconsistencies, six individuals were discarded due to a low call rate, and four duplicated or related patients were removed. PCA detected 30 individuals with a non-European ancestry who were excluded from the analysis; the plot of the first four PCs, explaining 16.3% of variance, are shown in (**Supplement Fig. 1B**). Finally, 48 patients did not have full NVS data or other covariates information; therefore, the GWAS was performed on 2052 patients.

The personal and clinical information of the patients is shown in Table 1. Males and females were equally distributed. Approximately one third of patients received morphine, another third was treated with fentanyl, 26% received oxycodone and only 5% took buprenorphine. The median morphine-equivalent dose was 110 MME. The most common types of cancer were gastro-enteric (∼ 18%), lung (∼ 18%) or breast (∼ 14%) cancer. More than two thirds of patients did not receive chemotherapy. Approximately 67% of patients were from the EPOS study, while 12% and 21% of subjects belonged to CERP and MOLO, respectively. Additionally, patients were mostly Italian, Norwegian, English and German. Of note, all non-Italian patients were in the EPOS study. The median value

Table 1
Clinical Characteristics of Patients Treated With Opioids Included in the GWAS for the Nausea-Vomiting Phenotype

Patient characteristic	NVS GWAS (n = 2052)
Age, years, median (range)	64 (18−91)
Sex, n (%)	
Female	1043 (50.8)
Male	1009 (49.2)
Opioid, n (%)	
Buprenorphine	103 (5.0)
Fentanyl	698 (34.0)
Morphine	711 (34.6)
Oxycodone	540 (26.3)
Opioid dose, MME, median (IQR)	110 (180)
Tumor diagnosis, n (%)	
Lung	356 (17.3)
Breast	296 (14.4)
Gastro-enteric	369 (18.0)
Pancreas	85 (4.1)
Prostate	209 (10.2)
Urinary traits	142 (6.9)
Head and neck	96 (4.7)
Gynecological	172 (8.4)
Other or unknown	327 (15.9)
Chemotherapy, n (%)	
Yes	646 (31.5)
No	1406 (68.5)
Study	
EPOS	1390 (67.7)
CERP	214 (10.4)
MOLO	448 (21.8)
Country of enrollment	
Switzerland	83 (4.0)
Germany	131 (6.4)
Denmark	12 (0.6)
Finland	28 (1.4)
Great Britain	207 (10.0)
Greece	4 (0.2)
Island	122 (5.9)
Italy	940 (45.8)
Lithuania	48 (2.3)
Norway	382 (18.6)
Sweden	95 (4.6)
Average NVS, median (IQR)	11.11 (33.33)

Abbreviations: MME = morphine milligram equivalents; IQR = interquartile range.

Table 2
Multivariable Linear Regression Between Clinical Variables and NVS Phenotype

Characteristic		Beta	*P*-Value
Sex (Male as reference)	Female	5.5	**3.9×10^{-5}**
Age		-0.12	**0.0090**
Study (EPOS as reference)	CERP	-10.9	**4.7×10^{-5}**
	MOLO	-15.6	**8.6×10^{-14}**
Opioid (Morphine as reference)	Buprenorphine	-6.2	**0.026**
	Fentanyl	-1.9	0.27
	Oxycodone	-0.44	0.79
Opioid dose (MME)		0.0027	0.12
Tumor site (Lung as reference)	Gastro-enteric	0.54	0.76
	Breast	-0.40	0.85
	Prostate	2.1	0.34
	Pancreas	-0.45	0.88
	Urinary tracts	0.96	0.69
	Head and neck	3.5	0.21
	Gynecologic	3.5	0.15
	Other	-2.1	0.26
Genotyping batch (I as reference)	II	2.9	0.13
	III	1.9	0.28
	IV	2.0	0.21
Chemotherapy (No as reference)	yes	1.4	0.29

Abbreviations: MME = morphine milligram equivalents.
Statistically significant associations are shown in bold.

for the NVS was 11.11 with an interquartile range of 33.33 and the mean value was 18.8 (standard deviation, SD = 24.9). The EORTC QLQ-C30 reference values for NVS in recurrent/metastatic cancer patients were lower than ours (median = 0, range: 0 − 16.7; mean = 13.1, SD = 22.5; https://www.eortc.org/app/uploads/sites/2/2018/02/reference_values_manual2008.pdf).

We searched for clinical variables significantly associated with the NVS phenotype, by performing a multivariable linear regression with sex, age, administered opioid, morphine-equivalent dose, chemotherapy, study, genotyping batch, and tumor type. We observed that females had higher NVS than male patients and that NVS slightly decreased with age. In addition, MOLO and CERP patients experienced less nausea-vomiting than patients in the EPOS group. Patients treated with buprenorphine had lower NVS than those receiving morphine. Finally, NVS did not significantly correlated with any of the other tested variables (Table 2). After performing a stepwise model selection (based on AIC), we selected the covariates to be used in the GWAS: age, sex, study, and dose (although the latter was not statistically significant; Supplemental Table 2).

For each patient, we genotyped 888,799 variants and 799,417 of them passed the Axiom Quality Controls pipeline; 13,767 variants were removed due to missing genotype data, and 946 and 387,355 variants were discarded due to Hardy Weinberg disequilibrium and low allele frequency (MAF < 1%), respectively. Finally, we removed 13,759 nonautosomal variants and 13,765 variants in extended LD. This dataset comprising 369,825 variants was used in REGENIE Step 1. After imputation and filtering by MAF < 2% and imputation score R^2 < 0.3, the dataset for Step two included 7,669,761 polymorphisms.

The GWAS in 2,052 patients was performed with REGENIE software between genotypes and NVS phenotype, using age, sex, study, and opioid morphine-equivalent dose as covariates. The results of this GWAS are shown in the Manhattan plot reported in Fig. 1. Although no associations reached the genome-wide statistical significance threshold (*P*-value < 5.0×10^{-8}), 65 variants were associated with NVS with a nominal *P*-value < 1.0×10^{-5} (Supplemental Table 3) The most significant association was observed for rs6562126, which was negatively correlated with NVS (*P*-value = 7.4×10^{-8}; beta = -4.34). This is an intronic

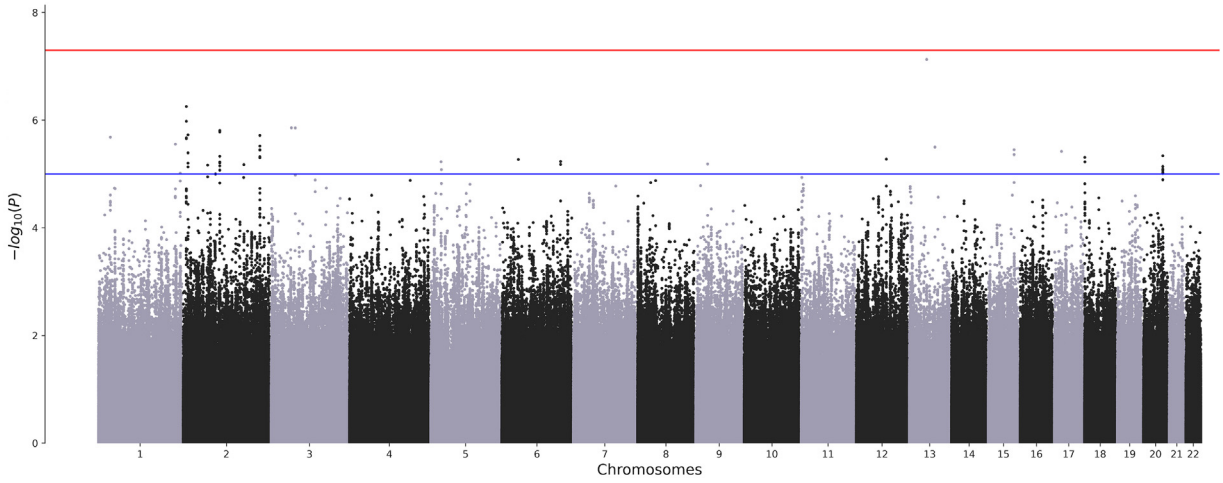

Fig. 1. Manhattan plot of the results from the GWAS with NVS and sex, age, study, and opioid morphine-equivalent dose as covariates. Each dot represents tested polymorphisms whose coordinates are determined according to their genomic position (GChr38, hg38 release) on the x axis, and *P*-values (-log10(*P*)) for their association with NVS on the y-axis. The horizontal red line represents the threshold of significance (*P*-value < 5.0×10^{-8}), while the blue line is a suggestive threshold at *P*-value < 1.0×10^{-5}.

variant of the long noncoding gene *LINC00378*, located on chromosome 13, approximately 118 kb downstream of the *TDRD3* gene. Among the other top-significantly associated variants, more than a half mapped to six loci, four of them on chromosome 2 (with four variants at six Mb, four at 10 Mb, 14 at 100 Mb, and six at position 219 Mb), one on chromosome 13, with four variants (19 Mb downstream the top-significant polymorphism), and one on chromosome 20, with 12 variants. Considering the two most numerous loci, the 14 variants on chromosome 2 mapped to the *NPAS2* gene. They all negatively correlated with NVS, meaning that an increasing number of minor alleles of these variants in the patients' genotypes was associated with less nausea/vomiting. The 12 variants on chromosome 20 mapped in an intergenic region, approximately 80 kb from the *ZNF217* gene. All these polymorphisms, instead, had positive beta coefficients, and thus their minor alleles were associated with high NVS.

Focusing on variants mapping in the *NPAS2* gene, we found, in the GTEx database, that six of them were *NPAS2* splicing quantitative trait loci (sQTLs), in the esophagus mucosa. In particular, an increasing number of minor alleles of these SNPs were reported to be associated with a high level of transcript splicing alteration. Table 3 reports the results from GTEx.

In addition, looking at the whole REGENIE output, we searched for variants previously reported for their possible association with nausea-vomiting in opioid-treated cancer patients.[6,8] Variants and their summary statistics, both from REGENIE analysis and the two published studies are shown in Supplemental Table 4. The rs12305038 variant, reported in,[8] showed nominal *P*-values < 0.05 also in the present study and concordant beta coefficients.

Discussion

In this pharmacogenomic study, on more than 2,000 advanced cancer patients treated with opioids to relieve pain, we looked for polymorphisms associated with nausea-vomiting side effect, one of these drugs' most common adverse events. Our GWAS identified 65 variants associated with NVS at a nominal *P*-value < 1.0×10^{-5}. The top-significant SNP, rs6562126, is an intronic variant of a long noncoding gene, *LINC00378*, and is near the Tudor domain containing 3 (*TDRD3*) gene. Unfortunately, no evidence for a role in nausea-vomiting has been reported thus far in the literature for both the variant and the genes where it maps. To our knowledge, the same is valid for most of the other identified variants.

Interestingly, 14 of the 65 variants mapped to the *NPAS2* (Neuronal PAS Domain Protein 2) gene, a transcription factor regulating circadian rhythm genes.[25] In particular, six of these variants are reported in the GTEx database as sQTLs of the *NPAS2* gene in the esophageal mucosa. Still, no evidence is available about any possible role of these SNPs in affecting *NPAS2* isoform function. Although any role of *NPAS2* or circadian genes in the regulation of opioid-induced nausea-vomiting has not yet been reported, the influence of circadian rhythm on gastrointestinal toxicity due to other cancer therapies (e.g., chemotherapy) is under investigation (as reviewed in[26]). Nonetheless, it has been reported that *NPAS2* might affect another common opioid side effect, i.e., the alteration of the sleep-

Table 3
Six Variants Associated With NVS and Mapping in the *NPAS2* Gene Were Previously Reported as *NPAS2* sQTLs, in GTEx.

Variant ID	Gene Symbol	Phenotype ID *	*P*-Value	NES	Tissue
rs7558747	NPAS2	chr2:100977799:100979559:clu_45742:ENSG00000170485.16	2.0×10^{-8}	-0.64	Esophagus - Mucosa
rs2289950	NPAS2	chr2:100977799:100979559:clu_45742:ENSG00000170485.16	1.9×10^{-6}	-0.52	Esophagus - Mucosa
rs4851393	NPAS2	chr2:100977799:100979559:clu_45742:ENSG00000170485.16	2.3×10^{-6}	-0.51	Esophagus - Mucosa
rs75107839	NPAS2	chr2:100977799:100979559:clu_45742:ENSG00000170485.16	2.3×10^{-6}	-0.51	Esophagus - Mucosa
rs3768985	NPAS2	chr2:100977799:100979559:clu_45742:ENSG00000170485.16	2.4×10^{-6}	-0.52	Esophagus - Mucosa
rs17025086	NPAS2	chr2:100977799:100979559:clu_45742:ENSG00000170485.16	2.7×10^{-6}	-0.51	Esophagus - Mucosa

Abbreviations: NES = normalized effect size.
*As reported in GTEx and defined as the intron (chr:start:end) and cluster of connected components (clu_) to which the intron belongs.

wake cycle. Indeed, it was observed that *NPAS2* absence exacerbated the harmful effect of fentanyl on sleep in mice.[27] Overall, our finding of an association between germline variants in a circadian gene and nausea-vomiting in cancer patients treated with opioids for cancer pain is very interesting, considering the complex relationship existing between circadian rhythm, pain, and opioids, as reviewed in[28]: indeed, both endogenous and exogenous opioids modify the circadian rhythm, pain sensitivity is altered by circadian rhythm dysregulation, and disrupted circadian rhythms can affect the efficacy of opioids. Further investigations are needed to understand the functional role of the *NPAS2* gene (and its germline variants) in opioid-induced nausea-vomiting and in the feedback loop between opioids and circadian rhythms.

Comparing our findings with the previously identified associations between germline variants and nausea-vomiting in opioid-treated cancer patients, unfortunately, we did not validate any of the SNPs reported by Laugsand *et al.*,[6] although more than 80% of the EPOS patients analyzed in that study were included in our dataset. However, in our study, EPOS patients were two thirds of the whole series, and possibly the remaining third (comprising CERP and MOLO patients) might be responsible for the lack of validation. Other reasons might reside in the differences between the analyses, in terms of the genetic model (dominant, recessive and codominant in[6] *vs.* additive, herein), stratification (by country and use of antiemetics in[6] *vs.* no stratification, in this study), and confounding factors included as covariates in the regression models. Of note, except for rs1672717, that in a dominant model with only nausea phenotype passed the Benjamini-Hochberg threshold set by authors at 10%, all the other variants were associated with NVS or nausea and vomit separately at low levels of significance (*P*-value < 0.01), as the authors themselves suggested that their analyses were explorative, and their results should be further evaluated in future studies.[6]

However, one SNP, rs12305038, identified by Colombo *et al.*,[8] was confirmed in our GWAS, with a nominal *P*-value < 0.05. In that study, a first discovery exome-wide study was carried out in the EPOS series (93% overlapping with EPOS patients in this study), and then, significant associations were tested in the CERP series (82% overlapping with CERP patients in this study) for validation. The rs12305038 variant was significant in both the discovery and validation series but with discordant beta values. Herein, we found that this SNP was associated with NVS at *P*-value = 0.015 and a beta coefficient concordant with that of the discovery study. Of note, several methodological differences between the two studies might have reduced the rate of result validation (e.g., DNA pooling strategy and exome sequencing *vs.* individual genome-wide genotyping by SNP-array; no adjustment for confounding factors in the exome data analysis) notwithstanding the overlap of patient series investigated in the two studies (> 90% of all the patients from the discovery and validation steps of Colombo's study were included in the genotyped series herein, of which they constituted the 78%). It might be interesting to compare our results with those from other studies reporting associations between germline variants and nausea-vomiting due to different etiologies (i.e., chemotherapy-induced or postoperative nausea-vomiting[29,30]) to explore the possibility that shared genetic factors might control similar phenotypes.

From a clinical point of view, our study has the limit of not having considered the use of other drugs (including antiemetics), as well as other psychological conditions (e.g. anxiety) that patients could have experienced, that possibly have affected the nausea and vomit symptoms. Unfortunately, these data were not fully available for all patient series. In addition, adding these further covariates to the GWAS could have reduced the power of the study. A major limitation of our study is the relatively small sample size, which did not allow for the identification of any locus associated with NVS at the genome-wide significance threshold. Nausea and vomiting are complex phenotypes for several reasons: they are quite subjective, influenced by external factors in addition to genetics, and the genetic factors involved are likely to be multiple and of very small effects. In our study, we used standardized questionnaires to collect data on nausea and vomiting and we followed standardized methods to calculate the NVS and thus to reduce potential bias. Unfortunately, as mentioned above, we could not control for some

confounders (for example, the use of other drugs) because we did not have complete information about them. Nevertheless, this is the largest GWAS on NVS in individually genotyped, opioid-treated cancer patients performed thus far. Collecting such a wide patient series, with quite homogeneously recorded data, was not easy and required several recruiting centers' collaborative efforts. A broader and independent series is needed to confirm and strengthen our results and to better dissect the genetic complexity of opioid toxicity. In addition, studies involving patients of different ancestries are required to ensure the generalizability of our findings.

Overall, our findings support a modest role of genetics in modulating nausea-vomiting due to opioid treatment. In our opinion, the hypothesis of a possible involvement of a circadian gene deserves further functional investigation. First, the role of the identified variants in affecting the splicing of *NPAS2* should be experimentally tested and the role of *NPAS2* splicing isoforms in the circadian cycle should be investigated, before exploring the mechanisms linking circadian rhythm and nausea-vomiting. In conclusion, our study encourages the scientific community involved in opioid research to put their efforts (and patient series) together to increase the statistical power of pharmacogenomic studies for opioid therapy and their clinical applicability.

Disclosures and Acknowledgments

This work was supported by the Fondazione AIRC per la ricerca sul cancro [MFAG 2019 - ID. 22950 - P.I. Colombo Francesca]. The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript. The authors declare no conflicts of interest.

Author Contributions

Conceptualization, F.C. and F.M; formal analysis, F. M., and F.C.; resources, A.T.C., M.SH., A.P., E.Z., F.S., P.K, S.K., O.C., M.C.M., M.C.P.; investigation—performing experiments, S.N. and F.C.; investigation—data collection, M.SH., C.B., A.P., E.Z., F.S., P.K, S.K., O.C., M.C.M., M.C.P.; data curation, C.B., M.SH., S.N., F.M, and F.C; writing—original draft preparation, F.M. and F.C.; writing—review and editing F.M., M.SH., A. P., P.K., and F.C.; funding acquisition, F.C. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The genotyping data are not publicly available due to privacy or ethical restrictions.

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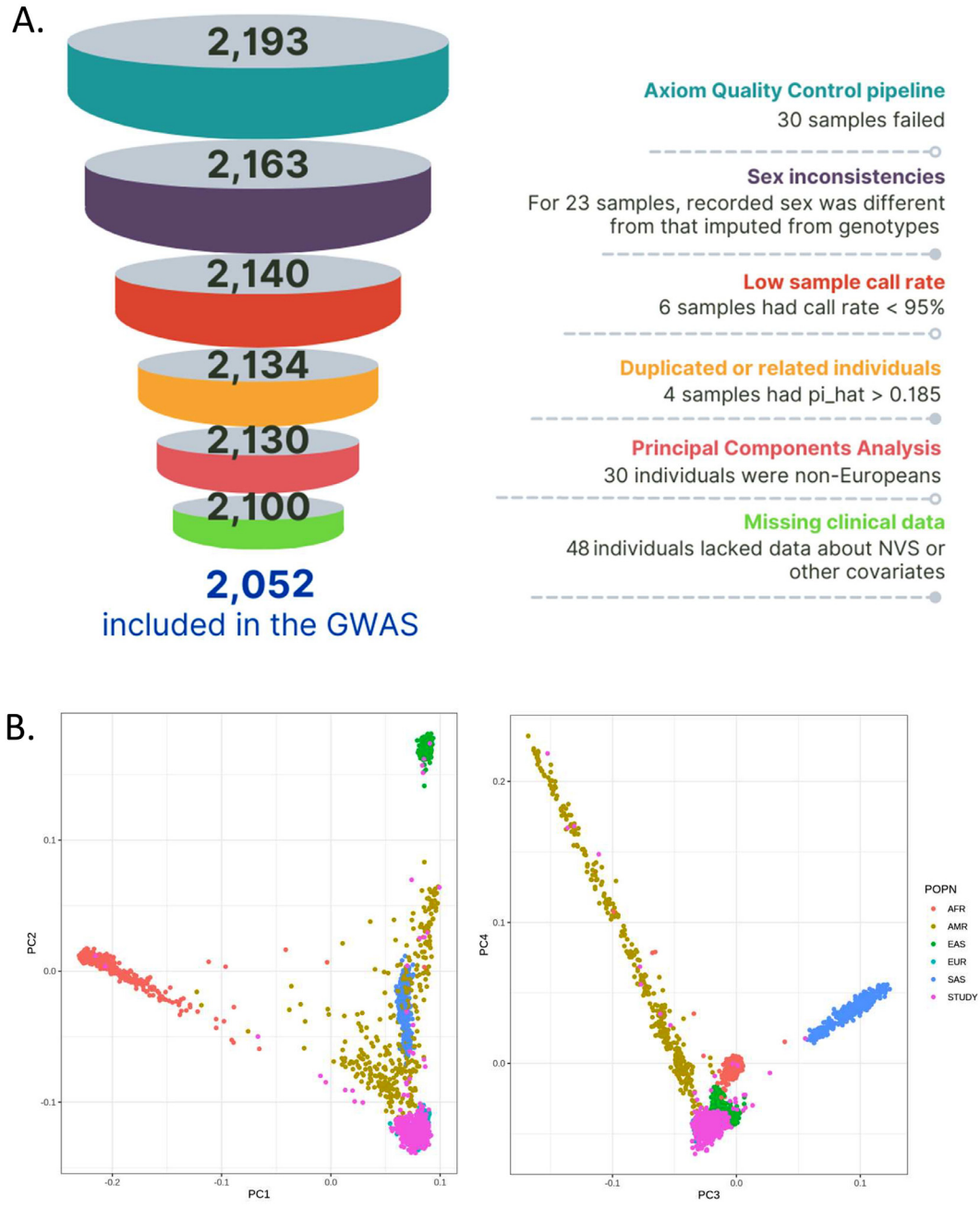
Appendix



Supplement Fig. 1. (a) Per-sample quality control steps. (b) Projection of the first four PCs of our samples (STUDY, pink dots) along those of 1563 individuals from the 1000 Genome reference populations.

Abbreviations: AFR = Africans; AMR = Americans; EAS = East Asians; EUR = Europeans; SAS = South-East Asians.

Supplement Table 1

Patient Inclusion Criteria for the Three Series

Study	Inclusion Criteria	Reference
CERP	Adults with histological or cytological evidence of advanced solid tumor, with a level of pain intensity greater than four (on a 0−10 numerical rating scale) requiring, for the first time, an analgesic treatment with step III WHO opioids (opioid-naïve patients); life expectancy >one month; no contraindications to fentanyl, oxycodone, buprenorphine or morphine	[9]
EPOS	Adults with malignant disease, who were using an opioid for moderate to severe pain (step III on the WHO treatment ladder for cancer pain).	[10]
MOLO	Adult patients with solid or metastatic tumor, with a level of pain intensity greater than four (on a 0−10 numerical rating scale) and a life expectancy >one month.	[11]

Supplement Table 2

Variables Selected as Covariates in the GWAS Model. Results of the Multivariable Linear Regression Model for NVS, Selected by a Stepwise Procedure Based on AIC, are Shown

		Beta	*P*-value
Age		-0.114	**0.010**
Sex (male as reference)	*female*	5.51	$\mathbf{2.5 \times 10^{-7}}$
Study (EPOS as reference)	*CERP*	-10.0	$\mathbf{2.5 \times 10^{-8}}$
	MOLO	-14.7	$\mathbf{<2.2 \times 10^{-16}}$
opioid morphine-equivalent dose		0.00239	0.14

Statistically significant associations are shown in bold.

Supplementary Table 3

Variants Most Significantly Associated With NVS (P-Value < 1.0 x 10^{-5}).

Variant ID	rsID[a]	Chr	Position (bp)[b]	Minor allele	MAF	P-Value	Beta	Variant Category	Gene Symbol[c]	Gene Start - End
chr13:60691995:C:A	rs6562126	13	60,691,995	A	0.72	7.42E-08	-4.3	ncRNA_intronic	TDRD3 (118,117 bp)	60,396,457−60,573,878
chr2:6214860:A:G	rs2044651	2	6,214,860	G	0.19	5.59E-07	4.7	intergenic	SOX11 (513,475 bp)	5,692,384−5,701,385
chr2:6210802:G:A	rs1562619	2	6,210,802	A	0.19	1.05E-06	4.6	intergenic	SOX11 (509,417 bp)	5,692,384−5,701,385
chr3:46866766:T:C	rs144291461	3	46,866,766	C	0.02	1.38E-06	12.0	intronic	MYL3	46,835,110−46,882,178
chr3:60120266:G:T	rs11715819	3	60,120,266	T	0.02	1.39E-06	11.9	intronic	FHIT	59,747,277−61,251,459
chr2:100967367:C:CA	rs899688453	2	100,967,367	CA	0.08	1.54E-06	-6.5	intronic	NPAS2	100,820,139−100,996,829
chr2:100979570:A:G	rs7558747	2	100,979,570	G	0.08	1.65E-06	-6.6	intronic	NPAS2	100,820,139−100,996,829
chr2:10455408:T:C	rs6721235	2	10,455,408	C	0.36	1.87E-06	3.5	ncRNA_intronic	ODC1 (7,081 bp)	10,439,968−10,448,327
chr2:219493864:G:A	rs16859981	2	219,493,864	A	0.27	1.92E-06	3.9	ncRNA_intronic	SPEG (235 bp)	219,434,843−219,493,629
chr1:30859255:C:T	rs41269495	1	30,859,255	T	0.12	2.07E-06	5.2	ncRNA_exonic	SDC3 (49,503 bp)	30,869,466−30,908,758
chr2:6212925:A:T	rs74474922	2	6,212,925	T	0.19	2.11E-06	4.5	intergenic	SOX11 (511,540 bp)	5,692,384−5,701,385
chr2:6213775:T:C	rs77772208	2	6,213,775	C	0.19	2.21E-06	4.5	intergenic	SOX11 (512,390 bp)	5,692,384−5,701,385
chr1:235354265:G:A	rs12120237	1	235,354,265	A	0.14	2.78E-06	4.8	intronic	GGPS1-TBCE	235,342,252−235,448,929
chr2:219491798:G:T	rs12473286	2	219,491,798	T	0.27	3.01E-06	3.8	exonic	SPEG	219,434,843−219,493,629
chr13:79720492:T:C	rs113196013	13	79,720,492	C	0.03	3.15E-06	10.9	intergenic	NDFIP2 (239,337 bp)	79,481,155−79,556,076
chr13:79727627:C:T	rs138202697	13	79,727,627	T	0.03	3.15E-06	10.9	intergenic	NDFIP2 (246,472 bp)	79,481,155−79,556,077
chr13:79757834:G:A	rs113650764	13	79,757,834	A	0.03	3.15E-06	10.9	intergenic	NDFIP2 (276,679 bp)	79,481,155−79,556,077
chr13:79774110:C:T	rs112441841	13	79,774,110	T	0.03	3.15E-06	10.9	intergenic	NDFIP2 (292,955 bp)	79,481,155−79,556,077
chr15:92363732:T:A	rs75516006	15	92,363,732	A	0.03	3.52E-06	9.4	intergenic	ST8SIA2 (30,149 bp)	92,393,881−92,468,728
chr2:219491357:G:A	rs4674405	2	219,491,357	A	0.27	3.52E-06	3.8	ncRNA_intronic	SPEG (2,272 bp)	219,434,843−219,493,629
chr2:219489643:C:T	rs56132883	2	219,489,643	T	0.27	3.60E-06	3.8	exonic	SPEG	219,434,843−219,493,629
chr17:15590028:G:A	rs3859258	17	15,590,028	A	0.03	3.78E-06	9.6	intronic	FBXW10B	15,565,483−15,619,704
chr2:10452054:C:T	rs2357551	2	10,452,054	T	0.33	4.05E-06	3.4	intergenic	ODC1 (3,727 bp)	10,439,968−10,448,327
chr15:92360112:A:T	rs76838139	15	92,360,112	T	0.03	4.34E-06	9.3	intergenic	ST8SIA2 (33,769 bp)	92,393,881−92,468,728
chr20:53689497:C:A	rs6022667	20	53,689,497	A	0.13	4.59E-06	4.9	intergenic	ZNF217 (79,590 bp)	53,567,071−53,609,907
chr2:100969022:T:C	rs4851393	2	100,969,022	C	0.09	4.70E-06	-6.1	intronic	NPAS2	100,820,139−100,996,829
chr2:219488291:G:A	rs875098	2	219,488,291	A	0.269737	4.77E-06	3.7	exonic	SPEG	219,434,843−219,493,629
chr18:565760:A:C	rs486633	18	565,760	C	0.79	4.89E-06	-4.1	intergenic	CETN1 (14,620 bp)	580,380−582,114
chr2:219486657:C:T	rs6726806	2	219,486,657	T	0.269493	4.98E-06	3.7	intronic	SPEG	219,434,843−219,493,629
chr12:78344470:G:A	rs688403	12	78,344,470	A	0.220273	5.27E-06	-4.0	ncRNA_intronic	NAV3 (131,460 bp)	77,324,641−78,213,010
chr6:35827923:ACT:A		6	35,827,923	A	0.02	5.36E-06	11.3	intronic	LHFPL5	35,797,206−35,845,397
chr6:146586022:G:A	rs62436209	6	146,586,022	A	0.11306	5.82E-06	5.2	intergenic	ADGB (12,945 bp)	146,598,967−146,815,462
chr5:23357704:T:A	rs76437223	5	23,357,704	A	0.02	5.93E-06	11.2	intergenic	PRDM9 (85,882 bp)	23,443,586−23,528,093
chr18:576287:C:G	rs507731	18	576,287	G	0.179581	5.94E-06	4.3	intergenic	CETN1 (4,093 bp)	580,380−582,114
chr2:100965046:A:G	rs17025086	2	100,965,046	G	0.08	6.02E-06	-6.1	intronic	NPAS2	100,820,139−100,996,829
chr2:100965272:T:C	rs3768985	2	100,965,272	C	0.08	6.02E-06	-6.1	intronic	NPAS2	100,820,139−100,996,829
chr2:100965783:AGCCCAGGCATGGGG:A	rs774019923	2	100,965,783	A	0.08	6.02E-06	-6.1	intronic	NPAS2	100,820,139−100,996,829
chr2:100966205:C:T	rs1867862	2	100,966,205	T	0.08	6.02E-06	-6.1	intronic	NPAS2	100,820,139−100,996,829
chr2:100968433:C:T	rs2289950	2	100,968,433	T	0.08	6.02E-06	-6.1	intronic	NPAS2	100,820,139−100,996,829
chr2:100965484:C:T	rs3768986	2	100,965,484	T	0.08	6.19E-06	-6.1	intronic	NPAS2	100,820,139−100,996,829
chr2:10456151:C:T	rs62127117	2	10,456,151	T	0.35575	6.24E-06	3.4	intergenic	ODC1 (7,824 bp)	10,439,968−10,448,327
chr9:22853832:A:G	rs117959313	9	22,853,832	G	0.03	6.50E-06	9.2	ncRNA_intronic	DMRTA1 (398,092 bp)	22,446,824−22,455,740
chr6:146609106:A:G	rs1115208	6	146,609,106	G	0.130117	6.61E-06	4.8	intronic	ADGB	146,598,967−146,815,462
chr2:167772826:A:G	rs77352129	2	167,772,826	G	0.03	6.67E-06	9.1	intronic	B3GALT1	167,293,001−167,874,045
chr2:56224617:T:C	rs114727015	2	56,224,617	C	0.0299708	6.80E-06	9.7	intronic	CCDC85A	56,183,990−56,386,172
chr2:100971180:A:T	rs75107839	2	100,971,180	T	0.09	7.07E-06	-5.9	intronic	NPAS2	100,820,139−100,996,829
chr20:53692448:T:A	rs141742171	20	53,692,448	A	0.13	7.22E-06	4.7	intergenic	ZNF217 (82,541 bp)	53,567,071−53,609,907

(*Continued*)

Supplementary Table 3
Continued

Variant ID	rsID[a]	Chr	Position (bp)[b]	Minor allele	MAF	*P*-Value	Beta	Variant Category	Gene Symbol[c]	Gene Start - End
chr20:53692564:G:A	rs73134247	20	53,692,564	A	0.13	7.22E-06	4.7	intergenic	ZNF217 (82,657 bp)	53,567,071−53,609,907
chr2:10456888:C:T	rs11685132	2	10,456,888	T	0.353801	7.33E-06	3.4	intergenic	ODC1 (8,561 bp)	10,439,968−10,448,327
chr20:53694463:C:T	rs6022675	20	53,694,463	T	0.13	8.14E-06	4.7	intergenic	ZNF217 (84,556 bp)	53,567,071−53,609,907
chr20:53695294:G:A	rs6022677	20	53,695,294	A	0.13	8.14E-06	4.7	intergenic	ZNF217 (85,387 bp)	53,567,071−53,609,907
chr20:53695395:T:C	rs7264081	20	53,695,395	C	0.13	8.14E-06	4.7	intergenic	ZNF217 (85,488 bp)	53,567,071−53,609,907
chr20:53695568:G:A	rs6022679	20	53,695,568	A	0.13	8.14E-06	4.7	intergenic	ZNF217 (85,661 bp)	53,567,071−53,609,907
chr20:53696291:A:G	rs16998327	20	53,696,291	G	0.13	8.14E-06	4.7	intergenic	ZNF217 (86,384 bp)	53,567,071−53,609,907
chr5:23958489:C:A	rs138045800	5	23,958,489	A	0.02	8.27E-06	11.0	ncRNA_intronic	PRDM9 (514,903 bp)	23,443,586−23,528,093
chr2:100967073:C:CTCAA	rs1573743506	2	100,967,073	CTCAA	0.08	8.45E-06	-6.0	intronic	NPAS2	100,820,139−100,996,829
chr2:100967124:A:T	rs7340468	2	100,967,124	T	0.08	8.45E-06	-6.0	intronic	NPAS2	100,820,139−100,996,829
chr2:100967496:G:C	rs59951797	2	100,967,496	C	0.08	8.45E-06	-6.0	intronic	NPAS2	100,820,139−100,996,829
chr2:100968489:A:G	rs35503589	2	100,968,489	G	0.08	8.45E-06	-6.0	intronic	NPAS2	100,820,139−100,996,829
chr20:53696033:C:T	rs66951711	20	53,696,033	T	0.13	8.64E-06	4.7	intergenic	ZNF217 (86,126 bp)	53,567,071−53,609,907
chr20:53698485:A:G	rs73283718	20	53,698,485	G	0.13	9.34E-06	4.7	intergenic	ZNF217 (88,558 bp)	53,567,071−53,609,907
chr20:53697154:G:A	rs7348874	20	53,697,154	A	0.13	9.44E-06	4.7	intergenic	ZNF217 (87,247 bp)	53,567,071−53,609,907
chr1:245880428:C:T	rs12041538	1	245,880,428	T	0.11	9.65E-06	5.3	intronic	SMYD3	245,749,342−246,507,312
chr20:53697273:G:A	rs7348908	20	53,697,273	A	0.13	9.78E-06	4.7	intergenic	ZNF217 (87,366 bp)	53,567,071−53,609,907
chr2:77359143:C:T	rs17014022	2	77,359,143	T	0.0319201	9.97E-06	9.2	intronic	LRRTM4	76,747,685−77,593,319

[a]rsID, variant ID from dbSNP
[b]According to GRCh38 human genome reference assembly
[c]Coding gene where the variants map or nearest coding gene (distance is indicated in brackets).Abbreviations: Chr = chromosome; MAF = minor allele frequency.

Supplement Table 4

Comparison Between the Summary Statistics of the Genetic Variants, Previously Reported by Colombo *et al.*[8] and Laugsand *et al.*[6] as Being Associated With Nausea and Vomiting in Opioid-Treated Cancer Patients, With Those Resulting in the Present GWAS

		previous studies		present study	
	variant ID	*P*-value	Beta	*P*-value	Beta
Colombo *et al.*[8]	rs36024412	0.04	-2.2	0.15	-1.1
	rs168107	0.001	-3.5	0.11	-1.3
	rs41269255	0.03	3.3	0.11	2.7
	rs9393888	0.01	-3.1	0.33	-1.2
	rs12305038	0.002	-3.3	**0.015**	-1.9
	rs11882256	0.02	2.8	0.21	1.1
	rs10405238	0.02*	5.5*	0.67	0.42
Laugsand *et al.*[6]	rs685550	0.006	-6.7	0.67	0.35
	rs1176744	0.005	4.1	0.33	-0.76
	rs1672717	0.004	5.8	0.48	-0.53
	rs165722	0.001	5.0	0.53	-0.46
	rs4680	0.002	4.4	0.51	-0.48
	rs4633	0.008	8.9	0.5	-0.44

*summary statistics from the validation study