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Original Article

Clinical, ethnic and genetic risk factors associated with postoperative nausea and vomiting in patients undergoing cancer surgery: a case-control study

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Abstract: Objectives: To identify the clinical, ethnic, and genetic factors contributing to the varying risks of postoperative nausea and vomiting (PONV) among a Brazilian population undergoing cancer surgery. Methods: A case-control study was conducted involving 152 patients who experienced vomiting and/or retching (cases) and 158 patients who did not report nausea, vomiting, or retching (controls) within 24 h following oncological surgeries. This study is registered as 'Genetic Polymorphism and postoperative nausea and vomiting (PONV)' under registration number NCT03627780 (https://clinicaltrials.gov/study/NCT03627780). Thirty-two polymorphisms associated with PONV predisposition and 15 polymorphisms for ancestry analysis were genotyped via real-time polymerase chain reaction (PCR) with customised TaqMan low-density array (TLDA) cards. Results: The C allele of the rs208294 polymorphism (*P2RX7* gene) was observed at a significantly higher rate in the control group than in the case group across the genotype (P=0.035), dominant (P=0.010) and allele (0.032) models, thus suggesting a protective effect against PONV. The genotype results for rs208294 were validated via Sanger sequencing, which confirmed the association in the dominant model (P=0.027). In a multivariate regression analysis that included rs208294 and clinical variables that were identified in the univariate analysis, only a prior history of PONV or motion sickness was observed to be a significant predictor of PONV (P<0.05). No association between rs208294 and PONV was detected in an external cohort consisting of 198 cases and 56 controls of Japanese descents (P>0.05). Additionally, ancestry analysis indicated a predominantly European genetic composition in the Brazilian cohort, which differed with the Asian composition of the independent validation cohort. Conclusions: A previous history of PONV or motion sickness was identified as being the strongest predictor of PONV in our analysis. Genetic association, ancestry and external validation analyses suggest that genetic factors for PONV may significantly differ across populations of different continental origins.

Keywords: Postoperative nausea and vomiting (PONV), polymorphisms, *P2RX7* gene, surgery, neoplasms

Introduction

The incidence of postoperative nausea or vomiting (PONV) in the general surgical population is approximately 30%, and it can increase to as high as 80% in high-risk patients who do not receive prophylaxis [1]. PONV significantly impacts postsurgical recovery, interferes with sleep and oral intake, and is one of the leading causes of unanticipated hospital admissions [2, 3].

The predisposition to PONV is multifactorial and involves factors related to anaesthesia, surgery

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or patient characteristics [4]. According to the Apfel score (which is a well-established predictive tool), factors such as female sex, nonsmoking status, a history of motion sickness and/or PONV and the use of opioids in the postoperative period are linked to an increased incidence of PONV [1]. Although these factors can help in identifying high-risk patients and guiding risk-based therapeutic interventions, they do not fully explain the variability in the occurrence and severity of PONV. Notably, a family history of PONV has been identified as a significant risk factor in paediatric patients, thus suggesting a genetic component of PONV susceptibility. Therefore, genetic factors contribute to susceptibility to PONV [5, 6].

Single nucleotide polymorphisms (SNPs) of specific candidate genes are implicated in the systemic pathways of nausea and vomiting, thereby potentially influencing individual responses to antiemetic drugs [7]. Multiple neurotransmitters are involved in the pathophysiology of PONV, and genes encoding membrane receptors, drug transporters, ion channels, metabolic enzymes, or structural proteins may be potential targets that are linked to PONV susceptibility [3]. Inherited factors are believed to play a significant role in the underlying susceptibility to PONV, as evidenced by increased rates of PONV observed across multiple generations within the same family [8] and a higher risk of PONV is observed among children with a family history of the condition [6].

However, the strength of the genetic associations has generally been modest, and these genetic factors alone cannot fully explain inter-individual differences in the severity of or baseline sensitivity to PONV [8]. Some studies have identified populations of African descent as exhibiting a reduced risk of PONV [9], whereas individuals of European descent appear to demonstrate a greater prevalence of PONV compared to American or Asian populations [10]. These studies were conducted in relatively homogenous populations, thereby prompting us to investigate whether similar findings could be observed in a more genetically diverse cohort, such as in the Brazilian population.

The present study aimed to explore whether clinical variables, ethnic backgrounds, and genetic polymorphisms are associated with PONV in a cohort of Brazilian patients undergoing oncologic surgery. This study represents the first investigation of genetic polymorphisms and their associations with PONV in a Brazilian population.

Methods

Study design

This was a single-centre, observational, case-control, prospective study that was conducted on patients who underwent elective oncological surgeries between June 2015 and March 2020. Participants were recruited as part of the flowchart for recruitment, sample collection, processing, and storage of the biobank of the Academic Biobank for Cancer Research Network at the University of São Paulo (USP), Center for Translational Research in Oncology (LIM24), Instituto do Câncer do Estado de São Paulo (ICESP), São Paulo, Brazil. Written informed consent was obtained from all of the participants before completing the epidemiological and clinical questionnaires. This article adheres to the STREGA Statement [11]. Anonymized data are available at doi.org/10.6084/m9.figshare.24638607.

Ethics

Ethical approval for this Biobank was granted by the Local Research Ethical Committee CEP - N° 031/12 of Universidade de São Paulo, São Paulo, Brazil, as well as the National Ethics Committee CONEP (BIOBANCO) - N° 023/2014 of the Ministry of Brazilian Health, Brasilia, Brazil. The ethics approval report for this projetct has been include in the Supplementary Material II.

Subjects

Patients were recruited after surgery. Patients who presented with vomiting and/or retching within the first 24 hours after surgery were defined as the patients. The controls included patients who did not experience nausea, vomiting or retching during the same time period. All of the patients were assessed by the postoperative pain care team, who interviewed the patients on a daily basis after surgery.

The inclusion criteria were as follows: patients classified as ASA physical status II to IV, ≥18-years-old, undergoing elective cancer sur-

geries, and with one or more risk factors according to the Apfel score [1].

Patients were excluded from the study if they refused to participate in the study, required prolonged intubation during the first 24 hours after surgery, or exhibited any cognitive impairment, confusion, agitation or delirium after surgery. Patients receiving antiemetic medications prior to surgery were also excluded from the protocol. Furthermore, patients with missing or inconsistent data were excluded from the final analysis.

Data sources

Data were collected from the Electronic Health Record (EHR). Researchers interviewed each patient on the first postoperative day to collect information and complete a data sheet.

Antiemetic administration was intraoperatively and postoperatively performed at the discretion of the anaesthesiologist and surgical team, with different teams selecting the types and dosages of the antiemetic drugs. The utilized antiemetics included dexamethasone, ondansetron, metoclopramide, dimenhydrinate, and droperidol. All of the data were recorded via Research Electronic Data Capture (REDCap v.9.8.5) [12].

Blood sample collection, processing and DNA extraction

Blood samples (15 mL) were collected from all of the patients, processed into buffy coats and stored at -80°C until use. Peripheral leukocyte DNA was extracted using a salting-out procedure [13]. The DNA concentration and purity were measured using a NanoDrop One/OneC Microvolume UV Spectrophotometer® (Thermo Fisher Scientific, USA). Only DNA samples with an absorption ratio (A260/A280) greater than or equal to 1.8 were used for the experiments. DNA integrity was verified via electrophoretic separation of nucleic acids on a 1% agarose gel. The samples were eluted in Tris-EDTA buffer solution and stored at 4°C until use.

Selection of the genetic variants associated with PONV

To identify the genetic variants associated with PONV, we reviewed the literature for polymorphisms related to drug metabolism, motion sickness, nausea, vomiting, and postoperative pain, with a focus on the variants that could represent a wide range of genes and metabolic pathways. Initially, we selected 96 SNPs in 24 genes based on peer-reviewed studies [7, 8, 14-21] (Table S1). We further analysed their genomic and clinical information using the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Pubmed (https://pubmed.ncbi.nlm.nih.gov/), Ensembl (https://www.ensembl.org/index.html), KEGG (https://www.genome.jp/kegg/), and dbSNP (https://www.ncbi.nlm.nih.gov/snp/) databases. Genes were classified by pathways using the Enrichr server (https://amp.pharm.mssm.edu/Enrichr/). Variants with frequencies lower than 15% in the 1000 Genomes, Genome Aggregation Database (gnomAD), and Online Archive of Brazilian Mutations (ABraOM) databases were excluded from the analysis. We prioritised variants with missense effects, which are more likely to exhibit pathogenic implications, or those located in untranslated regions (UTRs) with potential regulatory impacts. At least one variant was included in each gene or metabolic pathway, and variants without available TaqMan® assays were excluded from the analysis. Table S2 lists the 32 polymorphisms in 23 genes that were analysed in association with PONV. For missense polymorphisms, we provide additional information on their functional impacts, as predicted via PolyPhen (Polymorphism Phenotyping v2) and SIFT (Sorting Intolerant from Tolerant) software. PolyPhen classifies missense variants as benign, probable, or possibly damaging, whereas SIFT classifies them as tolerated or deleterious.

Selection of the ancestry-informative markers (AIMs)

For the ancestry analysis, we searched for polymorphisms with distinct frequencies in four main populations (Admixed American, European, Eastern Asian, and African) [22-25] to assess all of the self-reported racial groups in our study (“white”, “brown”, “black” and “yellow”) [26]. We excluded variants with missense effects, previous associations with clinical conditions, or genotyping frequencies lower than 15% in the 1000 Genomes and gnomAD databases. A total of 15 AIMs were selected for ancestry analysis (Table S4).

SNP Genotyping using TaqMan® Low-Density Array (TLDA) cards

The samples were genotyped using customised TLDA cards (Thermo Fisher Scientific, USA). Details on card customisation, genotyping, and analysis are provided in the Tables S2, S4.

Sanger sequencing of the rs208294 polymorphism (P2RX7 gene)

The rs208294 polymorphism (*P2RX7* gene) genotyping results that were obtained using TLDA cards were technically validated via Sanger sequencing on the same set of samples. The detailed sequencing methodology is described in the Supplementary Material I.

Genotyping of rs208294 (P2RX7 gene) in an independent validation cohort

An additional cohort [27] of 254 Japanese adults (198 controls and 56 patients with PONV at 24 hours) was included for validation of the rs208294-PONV association observed in the Brazilian population. The general cohort characteristics have been previously published [27]. Genotyping details are included in the Supplementary Material I.

Ancestry analysis

We estimated the proportions of ancestral populations for each individual to compare these proportions with self-reported racial identities and case-control groups, as described in the Supplementary Material I.

Study size

For sample size calculation, we assumed a minimum minor allele frequency (MAF) of 15% for all of the genetic variants (based on data from the 1000 Genomes Project, Genome Aggregation Database (gnomAD) and Online Archive of Brazilian Mutations (ABRAOM)), a 30% prevalence of PONV occurrence [28], a potential genotype relative risk of 1.5, a 5% type I error rate, and 80% power. The Genetic Association Study (GAS) Power Calculator by CaTS, University of Michigan (accessed on December 2017 at the following link: *https://csg.sph.umich.edu/abecasis/cats/gas_power_calculator/index.html)*, was used. The adequate sample size calculated a priori was 152 cases and 145 controls.

Statistical analysis

Statistical analyses were performed using SPSS® v25.0, with a significance threshold of <0.05. The allele and genotype frequencies were calculated via allele counting, and the Hardy-Weinberg equilibrium (HWE) for each polymorphism was tested via the χ^2 test. The analyses were based on four genetic models: genotype, dominant, recessive, and allele/multiplicative (details of these models are found in the Supplementary Material I). Chi-square tests or Fisher's exact tests were used to compare genotype and allele distributions between cases and controls, as well as differences in sociodemographic, clinical, and surgical variables. The Shapiro-Wilk test was used to assess data normality, and the nonparametric Mann-Whitney test was used to compare quantitative variables between groups. *P* values were adjusted for multiple comparisons via the Benjamini-Hochberg method with the "p.adjust" function in R v4.0.2. Univariate and multivariate binary logistic regression models were used to identify the PONV risk factors, and odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated. Carriers of the wild-type genotype/allele were used as the reference group in the logistic regression models.

Results

We assessed 415 patients for eligibility in this study. Of these patients, 20 patients did not meet the inclusion criteria, five patients refused to participate, 17 patients lacked blood collections, ten patients had low-purity DNA samples, and 53 patients were excluded because they presented with only nausea symptoms. After enrolment, no patients were excluded from the study, thereby resulting in a final sample of 310 patients for analysis. This sample included 152 patients who experienced vomiting and/or retching and 158 controls who did not report of nausea, vomiting, or retching within the first 24 hours following oncological surgeries.

Description of the study population

Significant differences were observed between cases and controls in terms of age, sex, history of PONV or motion sickness, postoperative opioid use, Apfel score and chemotherapy-induced vomiting (P<0.05; **Table 1**). Compared with the control group, the case group was younger, pre-

dominantly female (86.8%), had a greater incidence of prior history of PONV/motion sickness and chemotherapy-induced vomiting, had higher Apfel scores [1] and had greater postoperative opioid use. The primary utilized intraoperative antiemetics were ondansetron and dexamethasone (**Table 1**). No statistically significant differences were observed in the types or combinations of administered antiemetics (such as dexamethasone, ondansetron, metoclopramide, dimenhydrinate, or droperidol) between the case and control groups (P> 0.05). No clinically significant differences were observed in the other analysed variables; therefore, they were not included in the multivariate analysis.

Genotype and allele frequency distributions and association with PONV

All 47 genetic markers were genotyped; after quality control (Figure S1, Supplementary Material I), 1.3% of the genotypes were excluded from the final analysis. **Table 2** provides detailed information on the 32 selected polymorphisms associated with PONV and their corresponding 23 genes, genomic coordinates (hg19), Hardy-Weinberg equilibrium (HWE) results, and minor allele frequency (MAF) distributions between cases and controls compared with frequencies from the 1000 Genomes, gnomAD and ABraOM databases. Raw *p* values of the chi-square test comparing frequencies between cases and controls, as well as adjusted *p* values after multiple testing corrections, are also presented. All of the SNPs conformed to HWE, except for rs33985936 (*SCN11A*), rs1176744 (*HTR3B*), rs16947 and rs1065852 (*CYP2D6*) (P<0.05; **Table 2**).

A significant association was observed between the rs208294 polymorphism (*P2RX7* gene) and PONV in the allele, genotype and dominant models (**Tables 2**, **3**), although this association was lost after multiple testing corrections (**Table 2**).

Via the TLDA methodology, 6.5% of the genotype data for the rs208294 polymorphism were excluded because of quality control criteria. Consequently, we decided to validate our findings by sequencing a 258 bp fragment that included this SNP. Via this method, we accurately genotyped all of the samples without any missing data and confirmed the association between rs208294 and PONV in the dominant model (**Table 3**).

Age, sex, history of PONV or motion sickness, postoperative opioid use, chemotherapy-induced vomiting, and the rs208294 polymorphism were significantly associated with PONV in the univariate analysis. However, in the multivariate regression models including all of the independently associated variables, only a history of PONV or motion sickness remained a significant predictor of PONV. The rs208294 polymorphism lost statistical significance in both the TLDA and sequencing results (**Table 3**).

For the other examined polymorphisms, we detected an association between the rs324420 polymorphism (*FAAH* gene) and PONV in the allele model, with the A allele being more prevalent in controls (29.9%) than in cases (22.7%) (raw *P* value =0.041). However, this association was not observed in the other genetic models (**Table 2**). Moreover, no significant associations were observed for the remaining polymorphisms (**Table 2** for the allele model; data not shown for the other genetic models).

Validation of the rs208294 (P2RX7 gene) association with PONV in the Japanese Cohort

We did not validate the association of the rs208294 polymorphism with PONV in the Japanese cohort across any of the investigated genetic models (Table S3, Supplementary Material I). The prevalence of the *P2RX7* polymorphism was lower in the Japanese sample (42.3%) than in the Brazilian sample (50.7%).

Ancestry-informative marker (AIM) results

We subsequently analysed 15 AIMs, which included intronic or intergenic regions and one synonymous variant (Table S4, Supplementary Material I). All of the analysed markers (except for rs2814778) were in accordance with HWE (Table S5, Supplementary Material I). This table also shows the MAF for the case and control groups and the MAF for each main population of the 1000 Genomes Project (African-AFR, American-AMR, Eastern Asian-EAS, and European-EUR).

Distinct profiles for each ancestral population were identified; however, there were no signifi-

Table 1. Comparison of the patient characteristics, postoperative nausea or vomiting (PONV) risk factors and surgical data between controls (N=158) and cases (N=152)

Variables	Controls (N=158)	Cases (N=152)	χ^2/U	p	OR (95% CI)	p#
Self-reported ethnicityδ						
White	88 (55.7%)	94 (61.8%)	1.921	0.589[a]	1 (Reference)	
Brown	48 (30.4%)	40 (26.3%)			0.78 (0.47-1.30)	0.340
Black	19 (12%)	17 (11.2%)			0.84 (0.41-1.71)	0.628
Yellow	3 (1.9%)	1 (0.7%)			0.31 (0.03-3.06)	0.317
Age (years)						
Mean (SD)	52.42 (13.11)	49.13 (13.29)	10,523	0.060[b]	0.98 (0.97-0.99)	0.030*
BMI						
Mean (SD)	26.97 (6.10)	28.01 (5.55)	10,429	0.045[b,*]	1.03 (0.99-1.07)	0.120
Female Gender	117 (74.1%)	132 (86.8%)	8.020	0.005[a,*]	2.31 (1.28-4.17)	0.005*
History of PONV or motion sickness	34 (21.5%)	77 (50.7%)	28.62	<0.001[a,*]	3.74 (2.28-6.14)	<0.001*
Nonsmoking status	142 (89.9%)	142 (93.4%)	1.269	0.260[a]	1.60 (0.70-3.64)	0.263
Postoperative opioid use	132 (83.5%)	139 (91.4%)	4.400	0.036[a,*]	2.11 (1.04-4.27)	0.039*
Apfel						
1	10 (6.3%)	3 (2.0%)	35.527	<0.001[a,*]	1 (Reference)	
2	53 (33.5%)	24 (15.8%)			1.51 (0.38-5.98)	0.558
3	71 (44.9%)	61 (40.1%)			2.86 (0.75-10.88)	0.122
4	24 (15.2%)	64 (42.1%)			8.89 (2.25-35.08)	0.002*
History of previous chemotherapy	65 (41.1%)	67 (44.1%)	0.274	0.601[a]	1.13 (0.72-1.77)	0.601
Chemotherapy-induced nausea	37 (59.7%)	44 (67.7%)	0.882	0.348[a]	1.42 (0.69-2.93)	0.348
Chemotherapy-induced vomiting	20 (32.3%)	33 (50.8%)	4.472	0.034[a,*]	2.17 (1.05-4.45)	0.036*
Type of surgery						
Abdominopelvic surgery	107 (67.7%)	98 (64.5%)	0.780	0.677[a]	1 (Reference)	
Breast surgery	28 (17.7%)	33 (21.7%)			1.29 (0.73-2.28)	0.389
Other surgeries	23 (14.6%)	21 (13.8%)			0.99 (0.52-1.91)	0.993
Duration of surgery (min)						
Mean (SD)	238.7 (137.0)	241.5 (135.5)	11,741.50	0.735[b]	1.00 (0.99-1.02)	0.858
Fluid balance (mL)						
Mean (SD)	2,304.4 (1309.1)	2,269.4 (1186.8)	11,993.50	0.985[b]	1.00 (1.00-1.00)	0.805
Videolaparoscopy surgery	65 (41.1%)	48 (31.6%)	3.057	0.080[a]	0.66 (0.41-1.05)	0.081
Neuraxial Opioids use	102 (64.6%)	103 (67.8%)	0.356	0.551[a]	1.15 (0.72-1.85)	0.551
Intraoperative ondansetron use	150 (94.9%)	143 (94.1%)	0.110	0.740[a]	0.85 (0.32-2.26)	0.740

Intraoperative dexamethasone use	117 (74.1%)	114 (75%)	0.037	0.848[a]	1.05 (0.63-1.75)	0.848
PCA 24 h use	29 (18.4%)	28 (18.4%)	0	0.988[a]	1.00 (0.57-1.79)	0.988

Note: δ Official Brazilian Census categories [26]; BMI: body mass index; N: Number of individuals; SD: standard deviation; PCA: Patient controlled analgesia; PONV: postoperative nausea or vomiting; [a]Chi Square test (χ^2); [b]Mann-Whitney test (U); #Univariate Logistic Regression; OR: Odds Ratio; 95% CI: 95% confidence interval; *P<0.05.

Table 2. Description of the thirty-two selected polymorphisms in 23 genes associated with PONV, comparison of their Minor Allele Frequencies (MAF) between controls (N=158) and cases (N=152), description of the allelic frequencies available in other public databases and results of the Hardy-Weinberg Equilibrium (HWE)

dbSNP ID	Genomic coordinate (hg19)	Gene	Minor allele	χ^2 HWE (total sample)	p HWE (total sample)	MAF (%) Controls (N=158)	MAF (%) Cases (N=152)	χ^2	*p* value	adj *p* value	MAF (%) 1000 Genomes (global)	MAF (%) 1000 Genomes (AMR)	MAF (%) gnomAD (AMR)	MAF (%) ABraOM (Brazil)
rs3766246	chr1:46865671	*FAAH*	G	0.445	0.505	50	53	0.544	0.461	0.97	43.5	51.3	55.2	53
rs324420	chr1:46870761	*FAAH*	A	1.054	0.305	29.9	22.7	4.168	0.041*	0.584	26.2	35.2	34.5	26
rs2165870	chr1:239785420	*CHRM3*	G	0.928	0.335	75.3	76.6	0.15	0.699	0.97	77.5	66.7	60.4	68.6
rs685550	chr1:239924408	*CHRM3*	A	3.091	0.079	71.3	63.9	3.652	0.056	0.584	66.4	74.1	75.9	72.4
rs3755468	chr2:75382391	*TACR1*	C	3.148	0.076	62.3	64.6	0.358	0.55	0.97	56.1	57.1	59.4	62.4
rs17641121	chr2:155665752	*KCNJ3*	C	2.769	0.096	29.1	35.9	3.214	0.073	0.584	19.2	22.3	23.9	31.1
rs901865	chr3:11300707	*HRH1*	C	0.344	0.557	77.8	77	0.068	0.795	0.97	82.3	86.7	90.1	80.9
rs33985936	chr3:38936134	*SCN11A*	T	6.9	0.009*	19.9	20.1	0.002	0.968	0.997	15.4	22.5	24	20.1
rs11709492	chr3:38945984	*SCN11A*	T	0.013	0.91	25.9	24	0.31	0.578	0.97	26.4	13.8	16.8	24.3
rs6280	chr3:113890815	*DRD3*	T	1.665	0.197	54.5	52.6	0.207	0.649	0.97	51.4	57.3	54.9	55.5
rs6443930	chr3:183754294	*HTR3D*	C	0.019	0.891	52	52.8	0.044	0.834	0.97	53.4	45.8	46.3	53.7
rs6766410	chr3:183774762	*HTR3C*	A	0.518	0.472	38.5	39.5	0.057	0.811	0.97	46.5	52.7	53.8	41.9
rs6807362	chr3:183778010	*HTR3C*	C	0.171	0.679	49	50.4	0.107	0.743	0.97	55.6	67.1	59.5	54.8
rs1799971	chr6:154360797	*OPRM1*	G	0.082	0.775	13	13.5	0.035	0.851	0.97	22.3	20	21.5	14.9
rs72552763	chr6:160560881	*SLC22A1*	-	0.898	0.343	17.1	15.7	0.227	0.634	0.97	11.8	28.8	21.7	NA
rs622342	chr6:160572866	*SLC22A1*	A	1.169	0.28	69.7	67.2	0.429	0.512	0.97	74.1	59.9	64.7	69.6
rs1800795	chr7:22766645	*IL6*	G	0.13	0.718	73.9	76.3	0.488	0.485	0.97	85.9	81.6	78.6	74.9
rs1045642	chr7:87138645	*ABCB1*	G	0.018	0.894	59.6	59.5	0	0.997	0.997	60.5	57.2	54.8	58.5
rs1072198	chr7:120327349	*KCND2*	T	0.171	0.679	66.6	64.8	0.212	0.645	0.97	67.5	68.2	71.9	65.2
rs2545457	chr8:140661285	*KCNK9*	A	1.485	0.223	62.3	64.9	0.437	0.509	0.97	64.2	68.9	65.8	63
rs1800532	chr11:18047816	*TPH1*	T	2.432	0.119	31.8	35.7	1.002	0.317	0.97	32.1	37.2	38.3	34.5
rs1800497	chr11:113270828	*DRD2*	A	1.669	0.196	24.4	27.3	0.697	0.404	0.97	32.6	31.1	44.9	24
rs3758987	chr11:113775275	*HTR3B*	C	1.263	0.261	28.8	32.2	0.865	0.352	0.97	32.7	35.9	32.8	31
rs1176744	chr11:113803028	*HTR3B*	C	6.015	0.014*	30	33.8	0.999	0.318	0.97	35.4	40.8	35.5	36.3
rs1062613	chr11:113846006	*HTR3A*	C	0.208	0.649	68.7	72.5	1.042	0.307	0.97	75.2	83.1	86.3	71.2
rs208294	chr12:121600253	*P2RX7*	C	0.013	0.909	54.9	46	4.598	0.032*	0.584	53	39	30.3	55.6

rs35364174	chr17:48731392	*ABCC3*	A	0.076	0.783	45.6	46	0.013	0.909	0.97	40	45.2	45.7	44.7
rs1978153	chr17:48737861	*ABCC3*	G	1.456	0.228	38	36.5	0.142	0.707	0.97	43.9	37.5	38.5	40.5
rs4633	chr22:19950235	*COMT*	T	0.002	0.967	37.6	39.5	0.234	0.629	0.97	37.2	38	40.5	41.2
rs4680	chr22:19951271	*COMT*	A	0.056	0.813	38.2	39.8	0.163	0.686	0.97	36.9	37.8	40.5	40.5
rs16947	chr22:42523943	*CYP2D6*	A	110.999	<0.001*	36.5	32.4	1.076	0.3	0.97	35.9	32.7	31.2	39.2
rs1065852	chr22:42526694	*CYP2D6*	A	15.424	<0.001*	16.4	16.1	0.013	0.909	0.97	23.8	14.8	12.3	17.6

Note: HWE: Hardy-Weinberg Equilibrium; N: number of individuals; chr: chromosome; -: deleted; MAF: Minor Allele Frequency (the minor allele was considered as the variant allele, different from the wild-type allele); adj: adjusted; AMR: Ad Mixed American; PONV: postoperative nausea or vomiting; NA: not available; *P<0.05.

Table 3. Association of the rs208294 polymorphism (*P2RX7* gene) and PONV by TaqMan Genotyping assay and technical validation by Sanger Sequencing, considering the total sample of controls (N=158) and cases (N=152) in four different genetic models

Genetic Model	Genotypes/ Alleles	TLDA genotyping (TaqMan Genotyping assay)						Genotyping by Sanger Sequencing					
		Controls N (%)	Cases N (%)	χ^2	P	OR (95% CI)	p#	Controls N (%)	Cases N (%)	χ^2	p	OR (95% CI)	p#
Genotype	TT	28 (18.3)	43 (31.4)	6.698	0.035*	1 (Ref)		28 (17.7)	43 (28.3)	4.98	0.083	1 (Ref)	
	TC	82 (53.6)	62 (45.3)			0.39 (0.13-1.18)	0.1	86 (54.4)	74 (48.7)			0.53 (0.18-1.55)	0.248
	CC	43 (28.1)	32 (23.4)			0.37 (0.11-1.20)	0.1	44 (27.8)	35 (23.0)			0.44 (0.14-1.41)	0.168
Dominant	TT	28 (18.3)	43 (31.4)	6.695	0.010*	1 (Ref)		28 (17.7)	43 (28.3)	4.9	0.027*	1 (Ref)	
	TC+CC	125 (81.7)	94 (68.6)			0.38 (0.13-1.09)	0.07	130 (82.3)	109 (71.7)			0.50 (0.18-1.38)	0.179
Recessive	TT+TC	110 (71.9)	105 (76.6)	0.849	0.357	1 (Ref)		114 (72.2)	117 (77.0)	0.949	0.33	1 (Ref)	
	CC	43 (28.1)	32 (23.4)			0.73 (0.31-1.71)	0.47	44 (27.8)	35 (23.0)			0.71 (0.31-1.63)	0.415
Allele	T	138 (45.1)	148 (54.0)	4.598	0.032*	1 (Ref)		142 (44.9)	160 (52.6)	3.672	0.055	1 (Ref)	
	C	168 (54.9)	126 (46.0)			0.80 (0.41-1.54)	0.5	174 (55.1)	144 (47.4)			0.70 (0.41-1.21)	0.202

Note: N: Number of individuals; #adjusted for gender, age, history of PONV or motion sickness, postoperative opioid use and chemotherapy-induced vomiting; OR: Odds Ratio; CI: Confidence Interval; Ref: reference; *P<0.05.

cant differences observed between the case and control groups in either the supervised or unsupervised approach (P>0.05; Figures S2, S3, Supplementary Material I). We observed slight differences between cases and controls, primarily in the percentage of the EUR component, which was greater in the case group (66% in the case group vs. 62% in the control group), and in the AMR component, which was lower in the case group compared with the control group (Figure S3, Supplementary Material I). Finally, we compared self-reported race with molecular ancestry and observed higher percentages of European, African or Asian components in individuals self-identifying as "white", "black" or "yellow", respectively (Figure S4, Supplementary Material I).

Discussion

We conducted the first case-control study aimed at identifying clinical, ethnic, and genetic differences associated with PONV in a sample of the Brazilian population undergoing cancer surgery. Patients were selected based on low-, moderate-, and high-risk factors for PONV, as defined via the Apfel criteria [1, 4]. We identified female sex, younger age, a prior history of PONV, postoperative opioid use, higher Apfel scores, and a history of chemotherapy-induced vomiting as significant risk factors for PONV. These findings align with well-established risk factors that have been reported in the literature [1, 4, 29].

We investigated 32 genetic variants across 23 genes associated with drug metabolism, motion sickness, nausea, vomiting, postoperative pain, and PONV susceptibility, whereby we selected variants with a minimum MAF of 15%, as reported in genetic databases. The inclusion of less common variants would have reduced the statistical power or required a substantially larger sample size. All of the SNPs except for rs33985936 (*SCN11A*), rs1176744 (*HTR3B*), rs16947 and rs1065852 (*CYP2D6*) conformed to HWE, thereby suggesting random allele segregation within the population [30]. Deviations from HWE can arise from genotyping errors, assortative mating, selection, population stratification, or random chance [30].

Genetic association analyses were performed using genotype, dominant, recessive and allele genetic models, which complement each other in reducing the likelihood of false negatives. This approach allowed us to better explore the influence of SNP alleles and their modes of inheritance on PONV [30]. We observed that the C allele of the rs208294 polymorphism in the *P2RX7* gene was more prevalent among controls than in cases across genotype, dominant, and allele models, thus indicating a potential protective effect against PONV. The genotypes of rs208294 were validated via Sanger sequencing, thereby confirming the association in the dominant model. However, after adjusting for clinical variables in a multivariate regression model, only a prior history of PONV or motion sickness remained a significant predictor of PONV.

P2RX7 is a purinergic receptor for ATP that plays a crucial role in gastrointestinal inflammation by increasing the production of proinflammatory mediators [31]. Surgical procedures [32] and subsequent postoperative ileus [33] can induce inflammation, thereby potentially exacerbating PONV. Interestingly, antiemetic agents such as dexamethasone, 5-HT3 receptor antagonists [34] and NK1 receptor antagonists [35] exhibit anti-inflammatory properties. As a result, these medications may alleviate PONV not by directly affecting the vomiting centre but by reducing inflammation [31].

The rs208294 polymorphism, which is a missense variant of the *P2RX7* gene, may influence PONV. The T allele encodes a tyrosine in place of a histidine (C allele), thus resulting in a moderate gain-of-function effect [36]. In contrast, the C allele of rs208294 has been linked to reduced levels of IL-12p40 in patients with localised aggressive periodontitis [37], which is a pattern that is similarly observed with dexamethasone administration [38]. Therefore, patients with the C allele may exhibit a lower incidence of PONV. Additional studies are needed to further elucidate the involvement of this polymorphism in PONV.

Despite the lack of statistical significance for rs208294 in the multivariate model, we proceeded to validate the association in an independent cohort of Asian patients comprising 198 cases and 56 controls, as previously described [27]. However, no association between rs208294 and PONV was identified in the Japanese cohort. This cohort was previously explored regarding genetic associations with PONV using a genome-wide association study

(GWAS) designed for the Asian population [39]. Moreover, a recent GWAS [40] involving another Japanese sample similarly revealed no association between rs208294 and PONV. Importantly, the genetic backgrounds of the Asian samples differ from those of our Brazilian cohort, which displayed a high proportion of European ancestry; this observation is consistent with prior studies on the Brazilian population [26, 41]. Given the high rate of genetic admixture in Brazil (but with a predominant European influence), PONV risk factors may significantly vary between populations with different ancestral compositions. This scenario underscores the importance of further research in diverse cohorts worldwide.

In addition to the *P2XR7* gene (rs208294), we also detected an association between the *FAAH* gene (rs324420) and PONV in the allele model, with further details available in the Supplementary Material I.

This study is the first to investigate 32 polymorphisms associated with PONV alongside 15 ancestry markers in a Brazilian population. Our selection included at least one SNP from the most relevant genes that have been previously implicated in PONV susceptibility. Recently, a large-cohort GWAS of over 60,000 individuals demonstrated a homogeneous distribution of potential PONV-related variants across all chromosomes, including genes involved in inflammatory and immune response pathways, such as P2RX7 [42]. Our findings are valuable for understanding the genetic underpinnings of PONV among Brazilian patients with cancer.

The clinical significance of the investigation of genetic variants associated with PONV predisposition is based on their potential to improve personalised anaesthesia and postoperative care. The identification of genetic markers for PONV risk can help clinicians to predict which patients are more likely to experience PONV, thus allowing for customised preventive strategies and improved management. This approach could lead to a reduced incidence of PONV, increased patient comfort, faster recovery times, and lower health care costs. Moreover, an understanding of the genetic basis of PONV may facilitate the development of targeted antiemetic therapies.

A limitation of our study was the relatively small sample size, which was partly due to our decision to include only patients who experienced vomiting or retching. Although this choice reduced the sample size, it enhanced the robustness of our findings by excluding patients with only nausea, which is a subjective symptom with distinct pathophysiological mechanisms from vomiting [32]. Additionally, our study did not record the constant use of postoperative antiemetics, which could have influenced the incidence of PONV.

In conclusion, a history of prior PONV or motion sickness emerged as the strongest predictor of PONV in our study. Further research is needed to explore the rs208294 polymorphism in the *P2XR7* gene and its association with PONV in larger, ethnically diverse case-control studies. Our genetic analyses, ancestry assessments, and external validation emphasise the idea that PONV risk factors may vary across population-specific risk factors.

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Disclosure of conflict of interest

None.

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Supplementary Material I

Methods

TaqMan® Low-Density Array (TLDA) card customization

We customized 384-well TLDA cards (Thermo Fisher Scientific, USA) for SNP genotyping. These cards enable simultaneous analysis of multiple samples and markers using TaqMan® assays. Each assay includes a primer pair to amplify the region of a specific SNP and two allele-specific TaqMan MGB probes with distinct fluorescent dyes (VIC or FAM channels) to distinguish between wild type and polymorphic alleles. We designed a panel consisting of 47 validated TaqMan® assays (32 SNPs previously associated with postoperative nausea and vomiting (PONV) and 15 ancestry-informative markers (AIMs)), plus a reference marker (Hs99999901_s1), totaling 48 markers. Each 384-well plate allowed the simultaneous analysis of eight samples. Details of the TaqMan® assays are listed in Tables S2 and S3. A file with the precise locations of each marker in the TLDA card was used as a reference in the SDS v2.4 software (Thermo Fisher Scientific, USA).

SNP genotyping using TLDA cards and quality control filtering

DNA samples were diluted with ultra-pure water to achieve a concentration between 10 and 15 ng/µL. Each sample was then mixed with TaqMan Genotyping Master Mix (Thermo Fisher Scientific, USA) in equal volumes (50.5 µL), and 100 µL of the resulting mixture was loaded onto the TLDA cards. To prevent batch biases, case and control samples were randomly distributed across each card. Following manufacturer recommendations, TLDA cards were centrifuged, sealed, and included a non-template control (NTC) sample in each set to monitor any contamination risk.

Amplification of TLDA cards was conducted on the 7900 HT Real-Time PCR equipment (Thermo Fisher Scientific, USA), following the manufacturer's protocol. Each TLDA card was read twice, once before and after amplification. The pre-amplification read recorded the baseline fluorescence for each well, while the post-amplification read measured the final fluorescence levels. Genotypes were estimated by subtracting pre-amplification readings from post-amplification readings for each well on the card.

Data files from the 7900 HT Real-Time PCR system were analysed using TaqMan® Genotype Software v1.4.0 (Thermo Fisher Scientific, USA). A two-step quality control process was applied to increase result confidence and avoid misinterpretations. First, samples with low fluorescence in the reference marker (Hs99999901_s1) were discarded. Then, genotypes with a quality value below 95% were filtered out, retaining only high-quality genotypes.

Sanger sequencing of rs208294 polymorphism (P2RX7 gene)

Since the TaqMan SNP Genotyping assay (C___3019032_1_) used for genotyping the rs208294 polymorphism in this study was discontinued by the manufacturer (Thermo Fisher Scientific, USA), we validated our results using Sanger sequencing. Polymerase Chain Reaction (PCR) was performed with Platinum™ II Taq Hot-Start DNA Polymerase (Thermo Fisher Scientific, USA) and specific primers for the P2RX7 gene, amplifying a 258bp region containing the polymorphism of interest (rs208294).

The primer sequences were as follows: Forward Primer 5'-CAGTTCTTTCACATCTGTGGTTCTACG-3' and Reverse Primer 5'-GGTAGGACCCAGGACTTTGC-3'. These primers, commercially available from Thermo Fisher Scientific, USA, have catalogue numbers A15629 and A15630, respectively. The amplicons were purified using ExoSAP-IT™ Express PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA) to remove excess primers and dNTPs. Sanger sequencing was conducted using the BigDye Terminator v3.1 Cycle sequencing kit (Thermo Fisher Scientific, USA) on an ABI 3730 analyser (Thermo Fisher Scientific, USA).

The quality of the sequencing data and visualization of the electropherograms (in .ab1 format) were verified using SnapGene Viewer v5.2 software. We also used this software to compile the sequences into a FASTA file, which was subsequently annotated using ClustalX software by aligning the FASTA file with a reference sequence.

Genotyping of rs208294 (P2RX7 gene) in an independent validation cohort

Genotyping was performed in an independent cohort composed of 254 Japanese adults [1] using a custom TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, USA). The assay utilized the following primers: 5'-CACCAGGCAGAGACTTCACA-3' and Reverse primer 5'-CCTATAGGAATTCAGACCGGAAGGT-3'. The probe was labeled with VIC/FAM fluorescent dyes and designed to detect the sequence CTGGTTCCCTTCAT[A/G]CACTA, following the manufacturer's instructions.

Ancestry analysis

To evaluate the effectiveness of our molecular ancestry panel in classifying individuals, we downloaded genotypic data from the 1000 Genomes Project for the 15 ancestry-informative markers (AIMs) under investigation. Populations included Admixed American (AMR), African (AFR), European (EUR), and East Asian (EAS). An unsupervised analysis was conducted using STRUCTURE software v2.3.4 [2], simulating four potential populations and employing a Markov Chain Monte Carlo (MCMC) model with 100,000 interations and a burn-in of 100.000 samples. This procedure was subsequently repeated to compare our case and control groups.

Additionally, a supervised analysis was conducted using Admix95 software [3] to evaluate frequencies in our sample and the four ancestral populations (AMR, EUR, EAS and AFR). This enabled us to estimate the percentage of each ancestral population in individual participants, which was then grouped by self-reported race and by case or control groups.

Genetic models considered for statistical analysis

The association between polymorphisms and PONV was analysed using four genetic models commonly used in association studies: genotype, dominant, recessive, and allele/multiplicative. Each model was based on the AA, AB or BB genotypes, with two alleles per marker: A (wild type) or B (polymorphic).

In the genotype model, AA vs AB vs BB frequencies were compared across study groups. The dominant model compared AA vs AB+BB frequencies, assuming a dominant effect of the polymorphic allele, where a single copy of the polymorphic allele is sufficient to produce the phenotype. The recessive model compared AA+AB vs BB frequencies, assuming a recessive effect, where two copies of the polymorphic allele are required for phenotype manifestation. Finally, the allele/multiplicative model compared the frequency of allele A vs B across study groups.

Discussion

In addition to examining the rs208294 in the *P2RX7* gene, we identified an association of the rs324420 variant in the *FAAH* gene with PONV, though only in the allele model. This particular variant (C385A) is a missense polymorphism that leads to a significant reduction in FAAH enzyme activity [4]. Previous studies have also noted an association between this SNP and refractory PONV which can result in extended stays in the anaesthesia recovery room [5]. Additionally, this SNP has been linked to pain sensitivity [6]. However, given that the association was observed solely in the allele model and was not replicated in the other genetic models in this study, further investigation or validation with a larger sample size will be necessary to confirm these findings.

Table S1. Summary of the variants previously associated with drug metabolism, motion sickness, nausea, vomiting, and postoperative pain searched for our study (96 variants in 24 genes), main references analysing these variants, and the enriched pathways provided by each gene list

Pathway	Gene	Qty	Polymorphisms	References
Anandamide metabolism	*FAAH*	5	rs4141964, **rs3766246**, **rs324420**, rs2295632, kgp12517369	[5]
Codeine and Morphine metabolism	*CYP2D6*	10	**rs16947**, rs35742686, rs1135824, rs3892097, rs5030655, rs5030867, rs5030865, rs5030656, **rs1065852**, rs5030863	[7, 8]
IL-10 anti-inflammatory pathway	*IL6*	1	**rs1800795**	[9]
Neuroactive ligand-receptor interaction	*CHRM3*	3	**rs685550**, rs10802789, **rs2165870**	[7, 10]
	DRD2	3	**Taq IA** (**rs1800497**), rs4648317, rs12364283	[7, 11, 12]
	DRD3	1	**rs6280**	[12]
	OPRM1	1	**rs1799971**	[7, 11]
	P2RX7	3	**rs208294**, rs208296, rs7958311	[11]
	TACR1	1	**rs3755468**	[7]
Serotonin and Melatonin biosynthesis	*TPH1*	1	**rs1800532**	[12]
Serotonergic synapse	*KCND2*	5	rs17376373, rs702414, rs802340, rs12706292, **rs1072198**	[11, 13]
	KCNJ3	11	rs6435329, rs11895478, rs3106653, rs3111006, rs12471193, rs7574878, rs12995382, rs13398937, rs2591157, **rs17641121**, rs4467223	[11, 13]
	SLC6A4	3	rs1176713, 5-HTTLPR, rs25531	[7, 12]
Tandem pore domain potassium channels	*KCNK9*	4	rs2542424, rs2014712, **rs2545457**, rs888349	[11, 13]
Transmembrane transport of Small Molecules	*ABCB1*	5	**rs1045642**, rs2032582, rs1128503, 3435C>T, 2677G>T/A	[7, 8]
	ABCC3	7	**rs35364174**, rs4148412, rs739923, rs733392, **rs1978153**, rs7216383, rs886493	[9]
	HTR3A	5	**rs1062613**, rs1176713, rs33940208, rs1985242, rs10160548	[7, 8, 12, 14]
	HTR3B	9	**rs1176744**, rs1672717, rs3782025, **rs3758987**, Tyr129Ser, Ala223Thr, -100_-102delAAG, c5+201_+202delCA, c6-137C>T	[7, 8]
	HTR3C	2	**rs6766410**, **rs6807362**	[7, 8]
	HTR3D	1	**rs6443930**	[7, 8]
	SLC22A1	4	rs34447885, rs2282143, **rs72552763**, **rs622342**	[15]
Tyrosine metabolism	*COMT*	5	**rs4680**, **rs4633**, rs165722, rs4818, rs6269	[7, 11, 12]
Inflammatory mediator regulation of TRP channels	*HRH1*	1	**rs901865**	[12]
Muscle contraction	*SCN11A*	5	**rs33985936**, rs13080116, rs11720988, **rs11709492**, rs11720013	[9]

Qty: quantity of variants per gene. Enriched pathways were analysed by the Enrichr server. The final 32 selected variants for genotyping analysis are indicated in bold.

Table S2. List of the 32 genetic variants selected to be associated with PONV in our study. Columns describe TaqMan genotyping assays, genomic details and consequences for each variant

Assay	dbSNP ID	Chr	Position (hg19)	Variant alleles	Gene	Gene name	Variant consequence	Biotype	Analyzed alleles	aa change	PolyPhen prediction	SIFT prediction
C___1897305_10	rs3766246	1	46865671	A/C/G	*FAAH*	Fatty Acid Amide Hydrolase	Intron variant; non-coding transcript variant	Protein coding; processed transcript	A/G			
C___1897306_10	rs324420	1	46870761	C/A	*FAAH*	Fatty Acid Amide Hydrolase	Missense variant; non-coding transcript exon variant	Protein coding; processed transcript	C/A	P/T	Benign	Tolerated
C___9774837_30	rs2165870	1	239785420	A/C/G	*CHRM3*	Cholinergic Receptor Muscarinic 3	Non-coding transcript variant; intron variant	Processed transcript	A/C			
C___1018542_10	rs685550	1	239924408	G/A/T	*CHRM3*	Cholinergic Receptor Muscarinic 3	Non-coding transcript variant; intron variant	Processed transcript; protein coding	G/A			
C__27474834_20	rs3755468	2	75382391	T/C	*TACR1*	Tachykinin Receptor 1	Non-coding transcript variant; intron variant	Antisense; protein coding	T/C			
C___3251251_10	rs17641121	2	155665752	T/C	*KCNJ3*	Potassium Inwardly Rectifying Channel Subfamily J Member 3	Intron variant; non-coding transcript variant	Protein coding; processed transcript	T/C			
C__25471612_10	rs901865	3	11300707	T/C	*HRH1*	Histamine Receptor H1	5 prime UTR variant	Protein coding	T/C			
C____428338_20	rs33985936	3	38936134	C/A/T	*SCN11A*	Sodium Voltage-Gated Channel Alpha Subunit 11	Missense variant	Protein coding	C/T	V/I	Benign	Tolerated
C____428349_10	rs11709492	3	38945984	C/T	*SCN11A*	Sodium Voltage-Gated Channel Alpha Subunit 11	Intron variant	Protein coding	C/T			
C____949770_20	rs6280	3	113890815	C/T	*DRD3*	Dopamine receptor D3	Missense variant	Protein coding	C/T	G/S	Benign	Tolerated
C__28960641_10	rs6443930	3	183754294	G/A/C/T	*HTR3D*	5-Hydroxytryptamine Receptor 3D	Missense variant; splice donor variant; intron variant	Protein coding	G/C	G/A	Benign	Tolerated
C__26004660_10	rs6766410	3	183774762	C/A/T	*HTR3C*	5-Hydroxytryptamine Receptor 3C	Missense variant	Protein coding	C/A	N/K	Benign	Tolerated
C__28948667_10	rs6807362	3	183778010	G/A/C	*HTR3C*	5-Hydroxytryptamine Receptor 3C	Missense variant	Protein coding	G/C	G/A	Benign	Tolerated
C___8950074_1_	rs1799971	6	154360797	A/G	*OPRM1*	Mu (μ) opioid receptor	Missense variant; intron variant; non-coding transcript exon variant; NMD transcript variant	Protein coding; processed transcript; nonsense mediated decay	A/G	N/D	Probably damaging; benign; possibly damaging	Deleterious; tolerated
C__34211613_10	rs72552763	6	160560881	ATGAT/AT	*SLC22A1*	Solute Carrier Family 22 Member 1	Inframe deletion; 3 prime UTR variant; NMD transcript variant	Protein coding; nonsense mediated decay	ATGAT/AT	MI/I		
C____928527_20	rs622342	6	160572866	C/A	*SLC22A1*	Solute Carrier Family 22 Member 1	Intron variant; NMD transcript variant	Protein coding; nonsense mediated decay	C/A			
C___1839697_20	rs1800795	7	22766645	C/G	*IL6*	Interleukin 6	Non-coding transcript Variant; intron variant	Antisense; Protein coding	C/G			
C___7586657_20	rs1045642	7	87138645	A/G/T	*ABCB1*	ATP binding cassette subfamily B member 1	Synonymous variant; non-coding transcript exon variant	Protein coding; processed transcript	A/G	I		

C___2291537_10	rs1072198	7	120327349	C/T	*KCND2*	Potassium Voltage-Gated Channel Subfamily D Member 2	Intron variant	Protein coding	C/T			
C__16255498_10	rs2545457	8	140661285	G/A	*KCNK9*	Potassium Two Pore Domain Channel Subfamily K Member 9	NMD transcript variant; intron variant	Nonsense mediated decay; protein coding	G/A			
C___8940793_10	rs1800532	11	18047816	G/T	*TPH1*	Tryptophan 5-hydroxylase 1	Intron variant; NMD transcript variant	Protein coding; nonsense mediated decay	G/T			
C___7486676_10	rs1800497	11	113270828	G/A	*DRD2*	Dopamine receptor D2	Missense variant	Protein coding	G/A	E/K	Benign	Tolerated
C__27512451_10	rs3758987	11	113775275	T/C	*HTR3B*	5-hydroxytryptamine (serotonin) receptor 3B	Intron variant	Protein coding	T/C			
C___7488596_1_	rs1176744	11	113803028	A/C	*HTR3B*	5-hydroxytryptamine (serotonin) receptor 3B	Missense variant	Protein coding	A/C	Y/S	Benign	Tolerated
C___7488465_10	rs1062613	11	113846006	T/C	*HTR3A*	5-Hydroxytryptamine Receptor 3A	5 prime UTR variant	Protein coding	T/C			
C___3019032_1_	rs208294	12	121600253	T/A/C/G	*P2RX7*	Purinergic receptor P2X 7	3 prime UTR variant; NMD transcript variant; missense variant; non-coding transcript exon variant; intron variant; 5 prime UTR variant	Nonsense mediated decay; protein coding; retained intron; processed transcript	T/C	Y/H	Probably damaging; possibly damaging	Deleterious
C___2568032_10	rs35364174	17	48731392	G/A	*ABCC3*	ATP Binding Cassette Subfamily C Member 3	NMD transcript variant; intron variant	Nonsense mediated decay; protein coding	G/A			
C__11935512_20	rs1978153	17	48737861	C/G/T	*ABCC3*	ATP Binding Cassette Subfamily C Member 3	Intron variant; NMD transcript variant	Protein coding; nonsense mediated decay	C/G			
C___2538747_20	rs4633	22	19950235	C/T	*COMT*	Catechol O-Methyltransferase	Synonymous variant; non-coding transcript exon variant; NMD transcript variant	Protein coding; retained intron; nonsense mediated decay	C/T	H		
C__25746809_50	rs4680	22	19951271	G/A	*COMT*	Catechol O-Methyltransferase	Missense variant; NMD transcript variant; non-coding transcript exon variant	Protein coding; nonsense mediated decay; processed transcript	G/A	V/M	Benign	Tolerated; deleterious
C__27102425_10	rs16947	22	42523943	A/G/T	*CYP2D6*	Cytochrome P450 Family 2 Subfamily D Member 6	Non-coding transcript variant; intron variant; NMD transcript variant; splice region variant; missense variant; non-coding transcript exon variant	Antisense; nonsense mediated decay; protein coding; retained intron	A/G	C/R	Benign	Tolerated
C__11484460_40	rs1065852	22	42526694	G/A	*CYP2D6*	Cytochrome P450 Family 2 Subfamily D Member 6	Non-coding transcript variant; intron variant; missense variant; non-coding transcript exon variant	Antisense; protein coding; retained intron	G/A	P/S	Possibly damaging; probably damaging	Deleterious

PONV, postoperative nausea and vomiting; dbSNP, Single Nucleotide Polymorphism Database; Chr, chromosome; UTR, untranslated region; NMD, nonsense-mediated mRNA decay; All information was obtained from the Ensembl database. PolyPhen (Polymorphism Phenotyping v2) and SIFT (Sorting Intolerant From Tolerant) software were used for in silico prediction of the functional impact on the final protein for the missense polymorphisms.

Table S3. Association between rs208294 (*P2RX7* gene) and PONV in the Japanese cohort (N=254)

Genetic Model	Genotype/Allele	Controls		Cases		χ^2	p
		N	%	N	%		
Genotype	TT	70	35.4	17	30.4	0.49	0.786
	TC	91	46	28	50		
	CC	37	18.7	11	19.6		
Dominant	TT	70	35.4	17	30.4	0.48	0.487
	TC+CC	128	64.6	39	69.6		
Recessive	TT+TC	161	81.3	45	80.4	0.03	0.872
	CC	37	18.7	11	19.6		
Allele	T	231	58.3	62	55.4	0.32	0.574
	C	165	41.7	50	44.6		

N, number of individuals; PONV, postoperative nausea and vomiting.

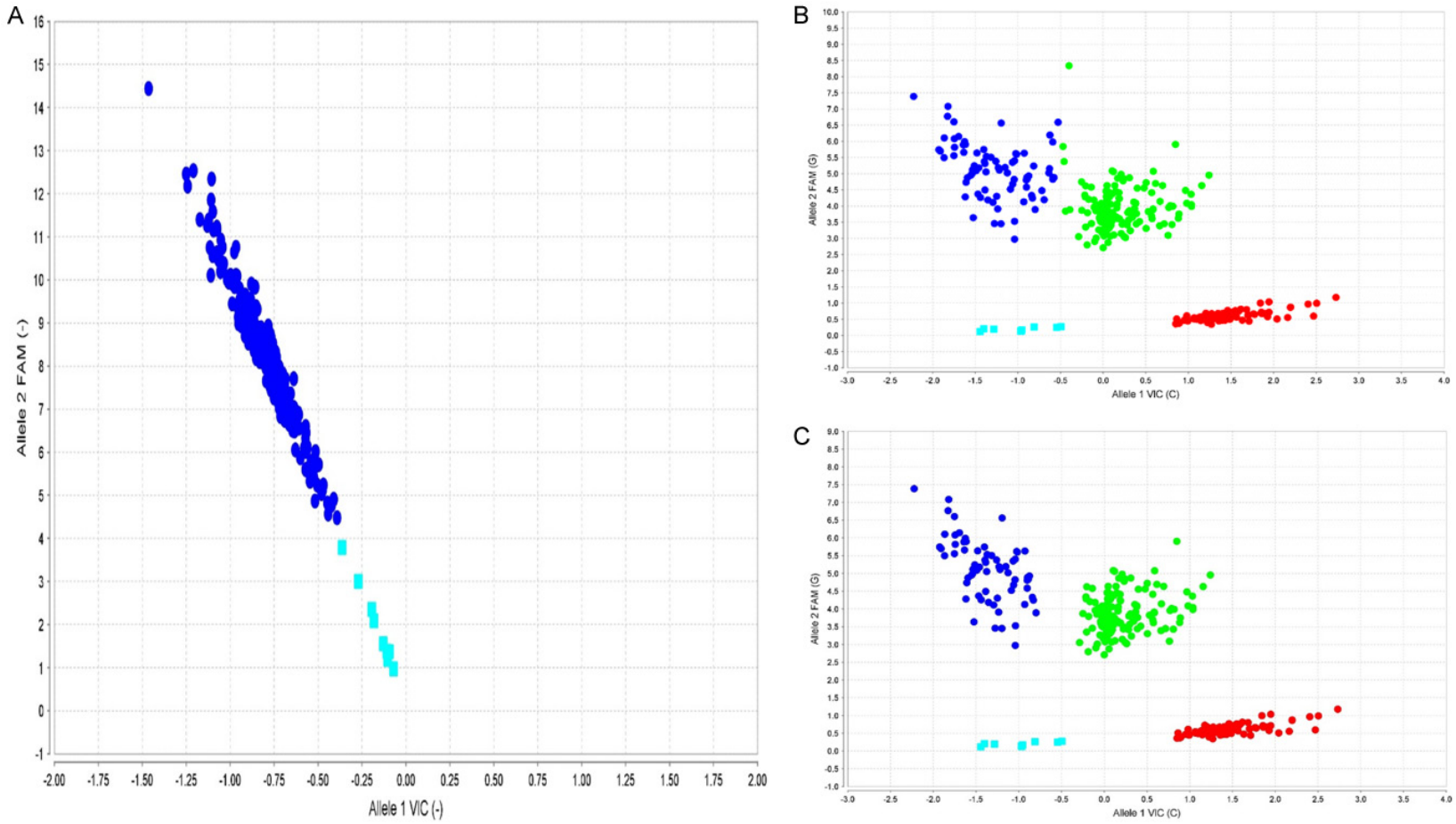

Figure S1. Two-step quality control assessment of TLDA results. (A) Verification of correctly amplified reference region in the FAM channel (blue dots). Genotyping results by TLDA for one polymorphism used as examples are shown before (B) or after (C) filtering for genotypes with a *Q*-value below 95%. Blue dots indicate wild-type homozygous, green dots indicate heterozygous, red dots indicate polymorphic homozygous and light blue squares represent non-template controls.

Table S4. List of the 15 variants selected for the ancestry investigation in our study. Columns describe TaqMan genotyping assays, genomic details and consequences for each variant and corresponding references

Assay	dbSNP ID	Chr	Position (hg19)	Variant alleles	Gene	Gene Name	Variant consequence	Biotype	References
C_176094026_10	rs140864	1	36391662	GAAGA/GA	*AGO1*	Argonauta 1	3 prime UTR variant	Protein coding	[16, 17]
C__15769614_10	rs2814778	1	159174683	T/C	*ACKR1*	Atypical Chemokine Receptor 1 (Duffy Blood Group)	Non-coding transcript variant; intron variant; 5 prime UTR variant	Antisense; protein coding	[16, 18]
C__11640969_10	rs1876482	2	17362568	G/A			Intergenic variant		[19]
C___8844929_10	rs952718	2	215888624	T/G	*ABCA12*	ATP Binding Cassette Subfamily A Member 12	Non-coding transcript variant; intron variant	Antisense; protein coding	[19]
C___8767848_10	rs1344870	3	21307401	T/A/G			Intergenic variant		[19]
C___3169933_1_	rs1363448	5	140783596	C/T	*PCDHGB4*	Protocadherin Gamma Subfamily B, 4	Intron variant; synonymous variant	Protein coding	[19]
C__26357333_20	rs2352476	7	137900133	T/G			Intergenic variant		[19]
C__12104266_10	rs285	8	19815189	C/T	*LPL*	Lipoprotein lipase	Intron variant	Protein coding	[16-18, 20]
C_189235823_10	rs714857	11	15974389	G/A			Intergenic variant		[19]
C___7566096_20	rs722869	14	97277005	C/G	*VRK1*	Vaccinia-related kinase 1	Intron variant; non-coding transcript variant	Protein coding; retained intron	[19]
C____489033_10	rs1129038	15	28356859	C/T	*HERC2, OCA2*	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 2	3 prime UTR variant; non-coding transcript exon variant; NMD transcript variant	Protein coding; retained intron; nonsense mediated decay	[16, 17]
C___2043758_10	rs735612	15	34076642	G/T	*RYR3*	Ryanodine receptor 3	Intron variant	Protein coding	[19]
C__12080106_10	rs1823718	15	74147244	A/G			Intergenic variant		[19]
C__11417706_10	rs1858465	17	51142920	A/T			Intergenic variant		[19]
C____958443_10	rs4884	19	45810035	A/G	*CKM*	Creatine Kinase, M-Type	Synonymous variant	Protein coding	[16-18, 20]

dbSNP, Single Nucleotide Polymorphism Database; Chr, chromosome; UTR, untranslated region; NMD, nonsense-mediated mRNA decay; All information was obtained from the Ensembl database.

Table S5. Description of the fifteen variants selected for the ancestry panel, comparison of their Minor Allele Frequencies (MAF) between controls (N=158) and cases (N=152), description of the allele frequencies available for the 1000 Genome Project in other public databases and results of the Hardy-Weinberg Equilibrium (HWE)

dbSNP ID	Genomic coordinate (hg19)	Gene	Minor allele	χ^2 HWE (total sample)	p HWE (total sample)	MAF (%) Controls (N=158)	MAF (%) Cases (N=152)	χ^2	p	MAF (%) 1000 Genomes (AMR)	MAF (%) 1000 Genomes (AFR)	MAF (%) 1000 Genomes (EUR)	MAF (%) 1000 Genomes (EAS)
rs140864	chr1:36391662	*AGO1*	-	1.638	0.201	10.13	9.54	0.06	0.806	30.3	11.27	1.19	64.98
rs2814778	chr1:159174683	*ACKR1*	C	23.306	<0.001*	19.62	20.39	0.058	0.81	7.8	96.37	0.6	0
rs1876482	chr2:17362568	-	A	8.916	0.003	11.08	9.21	0.591	0.442	23.1	0.23	7.75	77.48
rs952718	chr2:215888624	*ABCA12*	G	3.347	0.067	69.62	74.17	1.582	0.209	72.6	29.58	91.55	95.44
rs1344870	chr3:21307401	-	G	0.249	0.617	11.29	11.84	0.046	0.831	41.6	6.35	4.27	26.69
rs1363448	chr5:140783596	*PCDHGB4*	T	0.115	0.735	55.7	54.3	0.121	0.728	61.8	84.27	42.35	32.74
rs2352476	chr7:137900133	-	G	0.081	0.776	52.53	47.68	1.453	0.228	59.4	70.73	36.68	58.83
rs285	chr8:19815189	*LPL*	T	0	0.996	56.37	60.86	1.281	0.258	46.1	93.3	51.69	33.63
rs714857	chr11:15974389	-	A	0.05	0.823	22.44	25.34	0.704	0.401	22	77.23	6.56	61.31
rs722869	chr14:97277005	*VRK1*	G	0.892	0.345	14.01	16	0.476	0.49	34.3	12.56	10.34	83.13
rs1129038	chr15:28356859	*HERC2, OCA2*	T	2.366	0.124	23.42	27.3	1.237	0.266	20.3	2.8	63.52	0.1
rs735612	chr15:34076642	*RYR3*	T	0.253	0.615	59.81	57.57	0.322	0.57	43.8	41.15	66	95.63
rs1823718	chr15:74147244	-	G	0.55	0.458	44.62	42.43	0.301	0.583	46.4	21.86	49.4	4.96
rs1858465	chr17:51142920	-	T	0.293	0.588	69.43	73.68	1.375	0.241	85.2	17.1	85.39	73.71
rs4884	chr19:45810035	*CKM*	G	1.074	0.3	64.87	67.11	0.344	0.558	42.7	82.6	69.09	26.79

dbSNP, Single Nucleotide Polymorphism Database; HWE, Hardy-Weinberg Equilibrium; N, number of individuals; chr, chromosome; MAF, Minor Allele Frequency (the minor allele was considered as the variant allele, different from the wild-type allele); AMR, Ad Mixed American; AFR, African; EUR, European; EAS, Eastern Asian; *P<0.05.

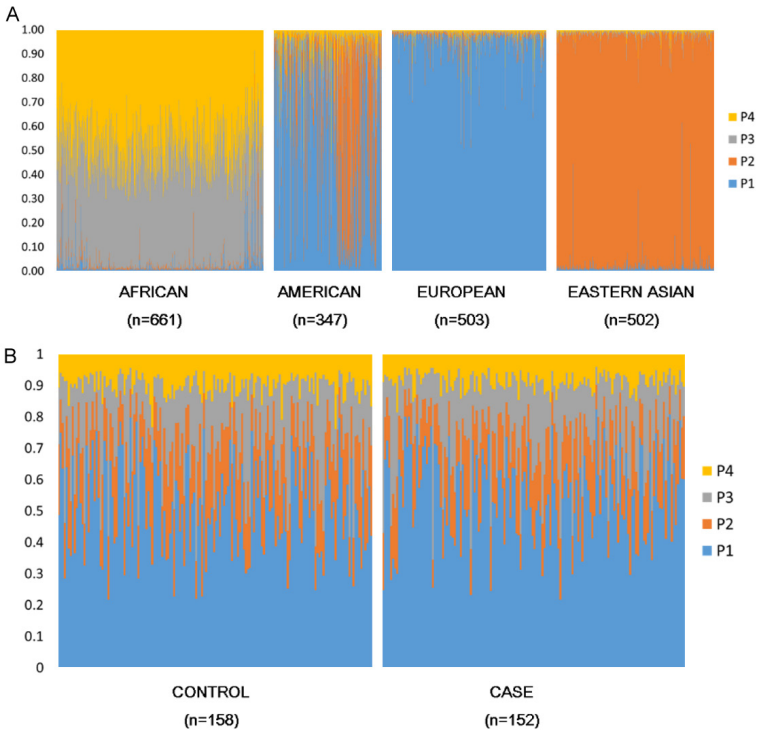

Figure S2. Ancestry features of the fifteen AIMs (ancestry informative markers) using putative populations. Colors (yellow, grey, orange, and blue) indicate putative populations, which were simulated to classify samples from the 1000 Genomes Project (A) and samples from our study (B). This ancestry panel can show differences among ancestral populations; however, it does not happen when comparing case and control groups in our study.

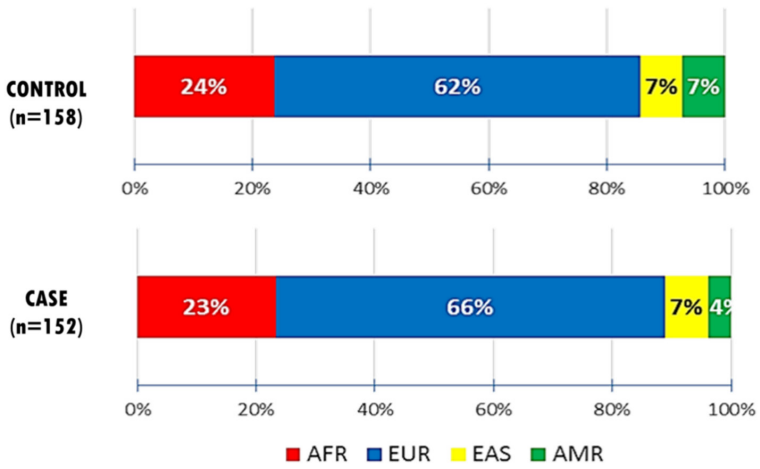

Figure S3. Estimation of ancestral components in case and control groups. After applying a supervised method, case and control groups show different percentages per component. Although ancestral contribution seems similar, the case group presents a higher European component than the control group. AFR: African component; EUR: European component; EAS: Eastern Asian component; and AMR: Ad Mixed American component.

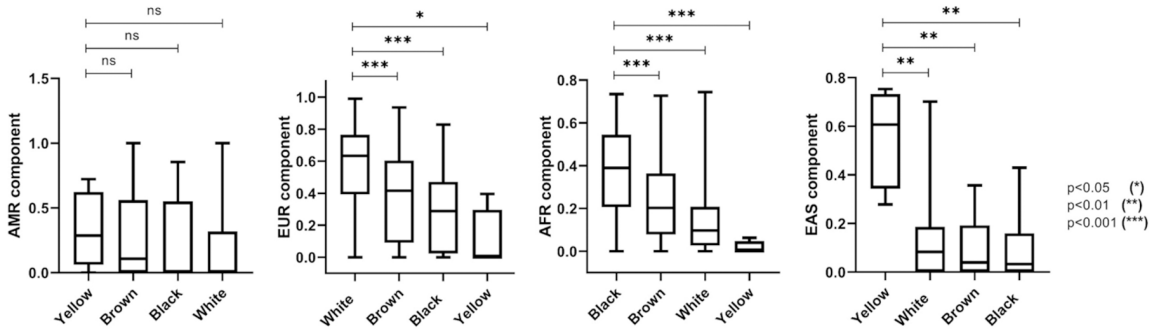

Figure S4. Ancestral percentages in people after race self-classification. Each bar plot represents the percentages of AMR, EUR, AFR or EAS components for all samples distributed by self-reported races. Asterisks indicate statistical differences, and the *p*-values were estimated using the Kolmogorov-Smirnov test. AFR: African component; EUR: European component; EAS: Eastern Asian component; and AMR: Ad Mixed American component; ns: non-significant.

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Supplementary Material II

USP - FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO - FMUSP

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Polimorfismo genético associado com a ocorrência de náuseas e vômitos pós-operatórios (NVPO) em pacientes submetidos a cirurgias oncológicas

Pesquisador: ANGELA MARIA SOUSA

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 5

CAAE: 78315717.2.0000.0065

Instituição Proponente: FUNDACAO FACULDADE DE MEDICINA

Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 3.081.643

Apresentação do Projeto:

Trata-se de Emenda (E2) apresentada para a Inclusão da pesquisadora e orientadora Profa. Dra. Maria José Carvalho Carmona (http://lattes.cnpq.br/2636111337875377) que orientará projeto de doutorada do pesquisador executante Alexandre Slullitel (http://lattes.cnpq.br/0352170203434442). Foi apresentado também relatório parcial com resultados obtidos no período. Foram estudados até o momento 273 pacientes, sendo 122 com náusea, 82 com náusea e vômito ou ânsia de vomitar. Os resultados parciais e análise estatística descritiva, permitiram concluir que o fator náusea e vômito induzido por quimioterapia (NVIQT) é um fator fator preditivo para NVPO. Além disso, os pacientes conhecidamente com maior chance de NVPO, como Apfel 3 e 4, quando fazem QT e apresentam náuseas pós QT, tem um risco muito maior de NVPO.

Os fatores causais dessa predisposição podem estar relacionados a modificações epigenéticas no sistema nervoso central, ou mecanismo de memória do centro do vômito, ou mesmo a modificações da microbiota intestinal.

Além disso, o levantamento dos SNPs relacionados a náuseas e vômitos mostram que até o momento, foram selecionados 50 variantes de um total de 17 genes, conhecidamente relacionados a NVPO em publicações anteriores. Foi iniciada a extração do DNA das amostras de sangue coletadas até o momento.

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Continuação do Parecer: 3.081.643

Objetivo da Pesquisa:

O objetivo primário do projeto é investigar se diferenças inter-individuais relacionadas a NVPO estão associadas marcadores genéticos representados por polimorfismos de nucleotídeos únicos (SNP) de genes candidatos nas vias de sinalização de NVPO. Objetivo Secundário: O objetivo secundário é investigar se diferenças inter-individuais

Avaliação dos Riscos e Benefícios:

De acordo com parecer n°2.417.052

Comentários e Considerações sobre a Pesquisa:

De acordo com parecer n°2.417.052

Considerações sobre os Termos de apresentação obrigatória:

Foi solicitada a inclusão da Profa. Dra. Maria José Carvalho Carmona como orientadora do pós-graduando Alexandre Slullitel, no entanto a Dra Maria Jose não foi inserida na equipe da Plataforma Brasil, somente no projeto completo. Foi apresentado também relatório parcial do período.

Recomendações:

Recomendo que seja incluída a pesquisadora Profa. Dra. Maria José Carvalho Carmona na equipe da plataforma e não somente no projeto completo.

Conclusões ou Pendências e Lista de Inadequações:

Aprovação com a recomendação de inclusão da orientadora de doutorado e pesquisadora Profa. Dra. Maria José Carvalho Carmona na equipe do projeto junto a Plataforma Brasil.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1263421_E2.pdf	23/11/2018 14:46:57		Aceito
Projeto Detalhado / Brochura Investigador	projetogenetica_AngelaSousa_AlexandreNVPO_23nov18.docx	23/11/2018 14:45:15	ANGELA MARIA SOUSA	Aceito
Declaração de Pesquisadores	carta_AngelaSousa_AlexandreNVPO_23nov18.pdf	23/11/2018 14:45:05	ANGELA MARIA SOUSA	Aceito
Declaração de Pesquisadores	AngelaSousa_TCLE_Biobanco_10abril2018.pdf	10/04/2018 12:18:22	ANGELA MARIA SOUSA	Aceito

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Continuação do Parecer: 3.081.643

Outros	intranet_AngelaSousa_NVPO_08MAR2018.pdf	09/03/2018 10:10:30	ANGELA MARIA SOUSA	Aceito
Declaração de Instituição e Infraestrutura	ParecerNP_ICESP_AngelaSousa_AlexandreNVPO.pdf	28/02/2018 11:03:51	ANGELA MARIA SOUSA	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	AnuenciaBIOBANCO_03Abr2017_AngelaSousa_AlexandreNVPO.pdf	28/02/2018 11:03:18	ANGELA MARIA SOUSA	Aceito
Folha de Rosto	FR_AngelaSousa_NVPO.PDF	28/09/2017 10:43:17	ANGELA MARIA SOUSA	Aceito
Projeto Detalhado / Brochura Investigador	projetogenetica_AngelaSousa_NVPO.pdf	28/09/2017 10:42:58	ANGELA MARIA SOUSA	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO PAULO, 13 de Dezembro de 2018

Assinado por:
Maria Aparecida Azevedo Koike Folgueira
(Coordenador(a))

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