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## Alterations in Patterns of Gene Expression and Perturbed Pathways in the Gut-Brain Axis Are Associated With Chemotherapy-Induced Nausea

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### Abstract

**Context**—Despite current advances in antiemetic treatments, approximately 50% of oncology patients experience chemotherapy-induced nausea (CIN).

**Objectives**—The purpose of this study was to evaluate for differentially expressed genes and perturbed pathways associated with the gut-brain axis (GBA) across two independent samples of oncology patients who did and did not experience CIN.

**Methods**—Oncology patients (n=735) completed study questionnaires in the week prior to their second or third cycle of chemotherapy (CTX). CIN occurrence was assessed using the Memorial Symptom Assessment Scale. Gene expression analyses were performed in two independent samples using RNA-sequencing (sample 1, n=357) and microarray (sample 2, n=352) methodologies. Fisher's combined probability method was used to determine genes that were differentially expressed and pathways that were perturbed between the two nausea groups across both samples.

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**Results**—CIN was reported by 63.6% of the patients in sample 1 and by 48.9% of the patients in sample 2. Across the two samples, 703 genes were differentially expressed and 37 pathways were found to be perturbed between the two CIN groups. We identified nine perturbed pathways that are involved in mechanisms associated with alterations in the GBA (i.e., mucosal inflammation, disruption of gut microbiome).

**Conclusions**—Persistent CIN remains a significant clinical problem. Our study is the first to identify novel GBA-related pathways associated with the occurrence of CIN. Our findings warrant confirmation and suggest directions for future clinical studies to decrease CIN occurrence.

### Keywords

chemotherapy; cancer; differential gene expression; pathway perturbation; nausea; mucosal inflammation; gut microbiome; gut-brain axis

## INTRODUCTION

Despite the use of guideline directed antiemetic regimens, nausea continues to be one of the most severe side effects of chemotherapy (CTX).<sup>1</sup> In fact, in our recent study,<sup>2</sup> 48% of patients reported CTX-induced nausea (CIN) prior to their second or third cycle of CTX. While a number of phenotypic characteristics are associated with unrelieved CIN,<sup>3</sup> less is known about the molecular characteristics associated with this symptom.

In a recent review,<sup>4</sup> we summarized the results of sixteen studies that evaluated for associations between genomic markers and the occurrence and/or severity of CTX-induced nausea and vomiting (CINV). The majority of the genes that were evaluated across these sixteen studies were related to the major mechanistic pathways for CINV (i.e., serotonin receptor pathway, drug transport pathway, and/or drug metabolism). In brief, none of the SNPs in the serotonin receptor gene and none of the alleles in the cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*) gene were associated with CIN occurrence. Three SNPs and two haplotypes in the ATP binding cassette subfamily B member 1 (*ABCB1*) gene showed inconsistent findings regarding their association with CIN occurrence.

Findings across these candidate gene studies are disappointing given that these genes were selected based on the major mechanisms for CINV. Therefore, a more exploratory, hypothesis generating approach is warranted to uncover additional mechanisms associated with the occurrence of CIN. One potential mechanism that warrants consideration is CTX-induced activation of the gut-brain axis (GBA).<sup>5,6</sup> Emerging evidence suggests that the administration of CTX results in mucosal inflammation<sup>7</sup> and disruption of the gut microbiome.<sup>8</sup>

In terms of its direct effects on the intestinal mucosa, CTX induces the synthesis and release of cytokines that results in mucosal inflammation and disruption of mucosal integrity along the entire gastrointestinal (GI) tract.<sup>5,6</sup> In addition, CTX-induced mucosal injury alters the gut microbiome and increases the release of additional inflammatory cytokines.<sup>5,6,8</sup> While findings from clinical studies have led a number of authors to hypothesize that CTX-induced activation of the GBA is associated with CIN,<sup>9,10</sup> no genomic studies were identified.

Therefore, to explore this hypothesis further, we evaluated for differentially expressed genes and perturbed pathways associated with the GBA across two independent samples of patients with and without CIN, after controlling for significant demographic and clinical characteristics.

## METHODS

This analysis is part of a longitudinal study that evaluated symptom clusters in oncology patients receiving CTX whose details are described elsewhere.<sup>2,11</sup> The methods are briefly described here.

### Patients and settings

Inclusion criteria were as follows: patients were ≥ 18 years of age; had one of four cancer diagnoses (i.e., breast, GI, gynecological, lung); had received at least one cycle of CTX; would receive at least two additional cycles of CTX; and were able to complete the study questionnaires in English. Recruitment occurred at two Comprehensive Cancer Centers, a Veteran's Affairs hospital, and four community oncology programs. Of the 2234 patients approached, 1343 provided written informed consent. The most common reason for refusal was that the patients were too overwhelmed with their cancer experience. For this paper, a total of 735 patients provided blood samples for the gene expression analyses (see Supplementary Figure 1).

### Study procedures

The Committee on Human Research at the University of California at San Francisco and at each of the study sites approved this study. Because of the stress associated with the receipt of CTX, patients were not recruited prior to the initiation of CTX. During their first or second cycle of CTX, eligible patients were approached in the infusion unit by a research nurse who discussed study participation. Patients completed questionnaires at home, six times over two cycles of CTX. The nausea groups for this analysis were created using data from the enrollment assessment (i.e., the assessment of nausea in the week prior to the patient's second or third cycle of CTX). Blood samples were collected at the time of enrollment. Medical records were reviewed for disease and treatment information.

### Instruments

**Phenotypic characteristics**—Demographic information was obtained using a self-report questionnaire. Functional status was assessed using the Karnofsky Performance Status (KPS) scale.<sup>12</sup> The occurrence, treatment, and functional impact of thirteen common medical conditions were assessed using the Self-Administered Comorbidity Questionnaire (SCQ).<sup>13</sup> Alcohol consumption was assessed using the Alcohol Use Disorders Identification Test (AUDIT).<sup>14</sup> A smoking questionnaire assessed smoking history.<sup>15</sup>

**Nausea assessment**—Nausea was assessed using a single item on the Memorial Symptom Assessment Scale (MSAS).<sup>16</sup> Patients indicated whether or not they had experienced nausea in the past week. Data from the enrollment assessment (i.e., occurrence

of nausea in the week prior to the patient's second or third cycle of CTX) were used to dichotomize the sample.

### **Coding of the emetogenicity of the CTX regimens and antiemetic regimens**

The coding of the emetogenicity of the CTX regimens and the antiemetic regimens were described previously.<sup>2</sup> Briefly, the Multinational Association for Supportive Care in Cancer (MASCC) guidelines<sup>1</sup> were used to classify each CTX drug in the regimen based on its emetogenic potential. Each antiemetic regimen was coded into one of four groups (see Table 1).

### **RNA sample preparation**

Whole blood was collected from patients at enrollment into PAXgene RNA stabilization tubes. Total RNA was isolated according to the manufacturer's standard protocol (Qiagen, USA) using the PaxGene Blood RNA Kit. All RNA samples demonstrated a RNA integrity number  $\geq 8$  and were retained for gene expression profiling.

### **Quantification of gene expression**

Of the 735 patients who provided a blood sample, for 375 patients gene expression was quantified using RNA-sequencing (RNA-seq) (i.e., sample 1) and for 360 patients using microarray (i.e., sample 2). Quantification of gene expression using RNA-seq, including library preparation, sequencing, and processing, were performed as previously described.<sup>17</sup> Quantification of gene expression, including microarray hybridization, quality control, and quantification, were performed as previously described.<sup>18</sup> All of these methods are described in detail in Supplementary File 1.

### **Data analyses**

**Demographic and clinical data**—Demographic and clinical data from the two patient samples (i.e., RNA-seq and microarray) were analyzed separately using SPSS Version 23 (IBM, Armonk, NY). Descriptive statistics and frequency distributions were calculated for all of the demographic and clinical characteristics. Differences in demographic and clinical characteristics between patients who did and did not report CIN were evaluated using Independent Student's t-tests, Mann Whitney U tests, Fisher's Exact tests, or Chi-square analysis.

Multiple logistic regression analysis was used to determine significant covariates for inclusion in the differential expression analyses. Only those characteristics that were significantly different in the univariate analyses between the nausea groups were evaluated in the logistic regression analyses. A parsimonious model was created using a backwards stepwise approach. Only those characteristics with a p-value of  $<0.05$  were retained in the final multivariate model.

**Differential gene expression and pathway perturbation analyses**—We performed differential gene expression analyses to quantify the differences in gene expression between patients who did and did not report CIN. To evaluate these results and interpret them in the

context of the GBA, we used pathway analysis to test for patterns in higher orders of biology.

The methods used to quantify differential gene expression in sample 1 (i.e., RNA-seq data)<sup>17,19</sup> and in sample 2 (i.e., microarray data)<sup>18,20</sup> were performed as previously described. Fisher's Combined Probability test was used to combine the differential gene expression tests from both datasets using the uncorrected p-values.<sup>21,22</sup> The two datasets (i.e., sample 1 and sample 2) were merged at the gene level using the ENTREZ gene identifier. The significance of the combined transcriptome-wide gene expression analysis was assessed using a strict false discovery rate (FDR) of 5% under the Benjamini-Hochberg (BH) procedure.<sup>23</sup> No minimal fold-change was evaluated using the p.adjust R function. These methods are described in detail in Supplementary file 1.

The differential gene expression results (i.e., p-value and log fold-change for all of the genes tested) were used to evaluate for perturbations in biological pathways. Pathway impact analysis (PIA) was used to determine the number of perturbed pathways. PIA was performed independently for sample 1 and sample 2. Fisher's Combined Probability test was used to determine the overall number of significantly perturbed pathways by combining the uncorrected p-values (i.e., pPERT) from the PIA tests for both samples.<sup>21,22</sup> Significance of the combined transcriptome-wide PIA analysis was assessed using a family wise error rate (FWER) of 1% under the Bonferroni method.<sup>24</sup> These methods are described in detail in Supplementary file 1.

## RESULTS

### RNA-seq performance

Of the 375 patients whose gene expression was quantified using RNA-seq (i.e., sample 1), 10 patients' results were determined to be outliers in the MDS plots and 8 patients had missing phenotypic data. Median library size was 9,273,000 reads. Genes with a threshold of 3.10 (10/L) in all of the samples were excluded, leaving 13,301 genes for analysis. The common dispersion was estimated as 0.179, yielding a biological coefficient of variation of 0.423 well within the expected value for clinical samples.<sup>25</sup>

### Microarray performance

Of the 360 patients whose gene expression was quantified using microarray (i.e., sample 2), four were excluded because of poor hybridization performance across all probes in the array; three patients were identified as outliers using distance array signal intensity distributions with ArrayQualityMetrics; and one patient had missing phenotypic data. All of the samples demonstrated good hybridization performance for biotin, background negative, and positive controls assays on the arrays. Limma was used for background correction, quantile normalization, and log<sub>2</sub> transformation.<sup>26</sup> Of the initial probes evaluated for quality (n=46,542), 1953 probes had insufficient expression measurements (Illumina detection p-value <0.05) and were excluded, leaving 44,589 probes for analysis.

### Differences in demographic and clinical characteristics

Of the 357 evaluable patients in sample 1, 227 (63.6%) reported nausea. Compared to the no nausea group, patients with nausea were significantly younger, had lower KPS and higher comorbidity scores, were fewer years from their cancer diagnosis, had a lower annual income, and were less likely to be employed. Compared to those patients without nausea, a lower percentage of patients with nausea had two types of cancer treatments and a higher percentage of patients received CTX on a 14 day cycle (Table 1). No significant differences were found between the two groups in the emetogenicity of the CTX regimens. While the overall test suggested that significant between group differences existed in the types of antiemetic regimens the patients received, none of the pairwise comparisons were significant.

Of the 352 evaluable patients in sample 2, 172 (48.9%) reported nausea. Compared to the no nausea group, patients with nausea had fewer years of education and a lower KPS score, and were more likely to be non-white, report child care responsibilities, have a lower annual income, have anemia or blood disease, and have depression. Compared to patients without nausea, a higher percentage of patients with nausea received CTX on a 14 day cycle; received highly emetogenic CTX; and were less likely to have received a steroid alone or a serotonin receptor antagonist alone compared to the no nausea group (Table 2).

### Logistic regression analyses

For sample 1, three variables were retained in the final logistic regression model (i.e., KPS score, CTX cycle length, type of prior cancer treatment) and were used as covariates in the gene expression analysis (Table 3). Patients who had a lower KPS score were more likely to be in the nausea group. Of the three pairwise contrasts that were done to examine the effect of CTX cycle length, only one contrast was significant. Compared to patients who received CTX on a 14 day cycle, patients who received CTX on a 21 day cycle had a 50% decrease in the odds of belonging to the nausea group. Of the six pairwise contrasts that were done to examine the effect of type of prior cancer treatment, only one was significant. Compared to patients who received only surgery, CTX, or RT, patients who received surgery and CTX, or surgery and RT, or CTX and RT had a 60% decrease in the odds of belonging to the nausea group.

For sample 2, four variables were retained in the final logistic regression model (i.e., having child care responsibilities, KPS score, emetogenicity of the CTX regimen, cancer diagnosis) and were used as covariates in the gene expression analysis (Table 3). Patients who had child care responsibilities and a lower KPS score were more likely to be in the nausea group. Of the three pairwise contrasts that were done to examine the effect of emetogenicity of the CTX regimen, only one contrast was significant. Compared to patients who received a CTX regimen with minimal or low emetogenicity, patients who received a CTX regimen with high emetogenicity were 3.40 times more likely to be in the nausea group. Of the six pairwise contrasts that were done to examine the effect of cancer diagnosis, two were significant. Compared to patients who had lung cancer, patients who had GI cancer were 5.00 times more likely to be in the nausea group. Compared to patients who had GI cancer,

patients who had gynecological cancer had a 64% decrease in the odds of belonging to the nausea group.

### Differentially expressed genes between the two nausea groups

For sample 1, clinical characteristics (i.e., KPS score, CTX cycle length, and type of prior cancer treatment) that differed between the groups were included in the final model for DE. For this gene expression analysis, 23 patients were excluded because of incomplete phenotypic data leaving 334 evaluable patients (n=213 with nausea, n=121 without nausea). While surrogate variable analysis (SVA) identified two surrogate variables for the RNA-seq data, neither was associated with CIN group membership. Both of these surrogate variables were included in the final model.

For sample 2, demographic (i.e., child care responsibility) and clinical (i.e., KPS score, emetogenicity of CTX, cancer diagnosis) characteristics that differed between the groups were included in the final model for DE. For this gene expression analysis, 58 patients were excluded because of incomplete phenotypic leaving 294 evaluable patients (n=140 with nausea, n=154 without nausea). SVA identified 23 surrogate variables for the microarray data. Four were associated with CIN group membership and were excluded. The remaining 19 surrogate variables were included in the final model.

In the combined analysis using Fisher's combined probability method, 18,124 genes were combined across both samples and 703 genes were significantly DE at a strict false discovery rate (FDR) of 5% [see Supplementary Table 1]. In order to evaluate the between CIN group differences in perturbations among genes that operate together in higher orders of biology, we performed a PIA of our GE data.

### Pathway impact analysis (PIA)

The PIA included all genes (i.e., cutoff free) and the results of the differential expression analyses (i.e., p-value and log fold change). For samples 1 and 2, assays with unique ENTREZ gene identifiers were used in the PIA (n=11,577 and n=20,216, respectively). In the combined analysis, using Fisher's combined probability method, 37 pathways were significantly perturbed using a strict family-wise error rate (FWER) of 1% (see Supplementary Table 2). We identified nine perturbed pathways associated with alterations in the GBA (Table 4).

### Supplemental Materials

Supplemental materials are available online (<https://doi.org/10.5281/zenodo.2527757>).

## DISCUSSION

While several lines of preclinical<sup>27</sup> and clinical<sup>9,10</sup> evidence suggest that CTX-induced activation of the GBA may result in a variety of GI symptoms (e.g., abdominal bloating), our pilot study is the first to present findings that suggest that a number of perturbed pathways associated with the GBA occur in patients with CIN. The GBA involves bidirectional communication between the central and the enteric nervous systems that is essential for

brain and gut function.<sup>6</sup> Mucosal inflammation and disruption of the gut microbiome can alter this bidirectional communication within the GBA.<sup>6</sup> Here, we discuss our findings regarding two potential mechanisms through which CTX can alter the function of the GBA and may result in CIN namely: mucosal inflammation<sup>6,9</sup> and disruption of the gut microbiome.<sup>6,8</sup>

### Mucosal inflammation

Because of its action on rapidly dividing cells, CTX damages the epithelial cells of the entire alimentary canal and results in mucosal inflammation.<sup>27</sup> This epithelial damage causes the release of reactive oxygen species (ROS) that activate nuclear factor- $\kappa$ B (NF- $\kappa$ B).<sup>7</sup> Activation of NF- $\kappa$ B in epithelial and immune cells results in the synthesis and release of inflammatory cytokines.<sup>7</sup> An amplification cascade ensues that results in the transcription of genes that encode for mitogen-activated protein kinase (MAPK) signaling molecules. Activation of the NF- $\kappa$ B signaling and MAPK signaling pathways,<sup>7</sup> as well as continued synthesis and release of inflammatory cytokines, results in the loss of mucosal integrity along the GI tract.<sup>7,27</sup>

Consistent with these mechanisms, we found perturbations in three pathways (i.e., cytokine-cytokine receptor interaction, MAPK signaling, NF- $\kappa$ B signaling) that have pre-clinical<sup>28-30</sup> and clinical<sup>31</sup> evidence to support their involvement in GI inflammation. In terms of cytokine-cytokine receptor interactions, in two preclinical studies, CTX-induced mucositis was associated with an increase in tumor necrosis factor-alpha (TNF- $\alpha$ ) immunostaining<sup>28</sup> as well as with increases in the expression of TNF- $\alpha$  and interleukin-6 (IL-6).<sup>30</sup> In terms of MAPK signaling, in a pre-clinical study of CTX-induced intestinal mucositis,<sup>29</sup> compared to rodents who did not receive irinotecan, the MAPK pathway was perturbed in the rats who received this CTX. Finally, in terms of the NF- $\kappa$ B pathway, in a clinical study of CTX-induced oral mucositis,<sup>31</sup> when compared to pre-treatment biopsies, increased oral mucosal staining for NF- $\kappa$ B was found in biopsies obtained following CTX.

Additional evidence that supports our hypothesis that CIN is associated with GI inflammation comes from our findings regarding perturbations in the chemokine signaling pathway. Chemokines are a family of small proteins that are involved in the recruitment and activation of leukocytes. Chemokines and their receptors mediate leukocyte trafficking in the gut and are associated with the development of intestinal inflammatory diseases.<sup>32</sup> In a pre-clinical study of 5-fluorouracil (5-FU)-induced gut epithelial damage,<sup>33</sup> compared to baseline, chemokine gene expression levels post-5-FU administration were significantly higher in the intestinal mucosa following the administration of 5-FU.

Given this initial evidence of associations between these four pathways and various types of mucosal inflammation, it is reasonable to hypothesize that these pathway perturbations may explain the occurrence of CIN observed in our study. While our findings support previously stated hypotheses regarding an association between mucosal inflammation and CIN,<sup>9,10</sup> additional research is warranted to evaluate the role of these mechanisms in CIN and other GI symptoms associated with the administration of CTX.



## Disruption of the gut microbiome

CTX-induced alterations of the gut microbiome can increase mucosal inflammation through a number of mechanisms including: alterations in the production and release of immunoglobulin A (IgA);<sup>5,8,34</sup> changes in cytokine synthesis;<sup>35,36</sup> increases in intestinal permeability;<sup>37</sup> and alterations in antigen processing and presentation.<sup>38,39</sup>

In terms of our finding regarding a perturbation in the intestinal immune network for IgA production, the gut microbiome regulates the synthesis of secretory IgA (sIgA) produced by mucosal B cells and in turn IgA regulates the composition of the gut microbiome.<sup>5,34</sup> Specifically, the intestinal immune network for IgA production pathway involves the differentiation of naïve B cells into sIgA producing plasma cells and their homing in the gut. The primary role of sIgA is to neutralize pathogens and toxins in the gut.<sup>40</sup> CTX-induced changes in the gut microflora cause a decrease in the levels of sIgA which results in GI inflammation.<sup>5</sup> Of note, in preclinical studies,<sup>41,42</sup> oral administration of specific bacterial species resulted in increased synthesis of IgA and decreased GI inflammation. In a recent study of oral mucositis in children receiving CTX for acute leukemia,<sup>43</sup> compared to a control group, the mean saliva concentrations of IgA were lower.

A second mechanism by which CTX-induced disruption in the gut microbiome can influence inflammatory processes is related to our findings of perturbations in the peroxisome-proliferation-activated receptor (PPAR) signaling and Th17 cell differentiation pathways. Certain resident microbiome species (e.g., *Bacteroids* and segmented filamentous bacteria (SFB)) can modulate signaling pathways involved in inflammation. For example, *Bacteroids*' activation of the PPAR signaling pathway in the intestinal epithelial cells (IECs) can result in decreased synthesis of pro-inflammatory cytokines and chemokines that are induced by the NF- $\kappa$ B signaling pathway.<sup>35</sup> In addition, emerging evidence from preclinical studies suggests that activation of PPAR $\alpha$  in the PPAR signaling pathway is involved in the regulation of inflammation, commensal homeostasis, and mucosal immunity in the gut.<sup>44</sup>

In terms of SFB, the presence of these bacteria in the gut microbiome is a prerequisite in the process of differentiation of interleukin 17 (IL-17)-producing T helper (Th17) cells into mature IL-17 producing Th17 cells.<sup>45</sup> Recent evidence suggests that the administration of cyclophosphamide favors the growth of SFB in the gut and enhances the differentiation of Th17 cells and associated increases in serum cytokines.<sup>36</sup> Given that both the PPAR signaling and Th17 cell differentiation pathways are associated with disruptions in the gut microbiome and GI inflammation,<sup>36</sup> their roles in CIN warrant investigation in future studies.

A third mechanism by which CTX-induced alterations in the gut microbiome may alter the functioning of the GBA is by influencing the synthesis of tight junction proteins.<sup>37</sup> CTX can increase intestinal permeability in two ways.<sup>37</sup> First, CTX-induced release of TNF- $\alpha$  downregulates the synthesis of tight junction proteins that results in increased epithelial permeability.<sup>46</sup> Second, CTX can decrease the numbers of bacteria that regulate the synthesis of tight junction proteins and increase the permeability of the epithelial lining of the GI tract.<sup>5,37</sup> Evidence from a number of clinical studies suggests that the administration

of 5-FU, doxorubicin, and mitomycin (FAM);<sup>47</sup> or oxaliplatin, folinic acid, and 5-FU (FOLFOX);<sup>47</sup> disrupts tight junctions and increases intestinal permeability.

In keeping with our finding of a perturbation in the tight junction pathway, recent evidence suggests that glutamine can decrease CTX-induced intestinal permeability. In fact, two systematic reviews provide evidence that supports the use of glutamine to prevent treatment-related mucositis in patients with cancer<sup>48</sup> and to decrease complications (e.g., mucositis, diarrhea) associated with colorectal cancer treatment.<sup>49</sup> However these findings need to be interpreted with caution because glutamine is known to be a major nutrient that contributes to the proliferation of cancer cells.<sup>50</sup>

A fourth mechanism by which CTX-induced changes in the gut microbiome can result in alterations in the GBA is related to our findings of a perturbation in the antigen processing and presentation pathway. The antitumor activity of CTX increases levels of tumor-derived peptide antigens (TDPAs).<sup>38</sup> Translocation of TDPAs, as well as the micro-organisms present in the gut, into the permeable intestine activates antigen presenting dendritic cells (APDCs) in the lamina propria.<sup>38</sup> APDCs adjust the adaptive immune response based on changes in the intestinal environment.<sup>36,38</sup> In addition, IECs function as antigen presenting cells and activate T cells in the lamina propria that are involved in downstream inflammatory processes.<sup>39</sup> Of note, and related to our finding of differential expression of *heat shock family protein D (Hsp60) membrane 1 (HSPD1)*, extracellular HSPD1 interacts with toll like receptors to present TDPAs to immune cells and induces the release of cytokines.<sup>51</sup> Activation of the antigen processing and presentation pathway in IECs and APDCs results in the release of inflammatory cytokines, which aggravates GI inflammation.<sup>38,39</sup> While Hsp60 is being investigated as a novel target to treat cancer,<sup>52</sup> its role in CIN warrants additional investigation.

### Limitations

While our study has numerous strengths including: a large sample size, stringent quality control procedures, strict criteria for differential gene expression and pathway perturbation selection, and the combination of results from independent tests across two samples, several limitations warrant consideration. First, we were not able to account for between group differences in annual household income in both logistic regression analyses because of a large amount of missing data. While we have indirect evidence from blood samples to support our hypothesis that CTX-induced changes in the GBA are associated with CIN, future studies are warranted that obtain tissue samples along the GI tract to provide direct evidence for this association. While our sample was large and representative of patients with CIN, our findings warrant confirmation in an independent cohort. Given that our phenotype and gene expression measurements were done prior to the patients' second or third cycle of CTX, additional research is warranted to determine if these changes in gene expression and pathway perturbations occur at other time points during the administration of CTX.

### Conclusions

In summary, our study is the first to report on associations between the occurrence of CIN and two mechanisms by which CTX can alter the function of the GBA (i.e., mucosal

inflammation, disruption of the gut microbiome). Findings from several clinical studies support an association between CTX-induced changes in the GBA and a number of GI symptoms.<sup>6,9,10</sup> While our findings suggest that additional research is warranted to evaluate the complex mechanisms that underlie the occurrence of CIN, they provide some preliminary insights into why unrelieved CIN remains a significant clinical problem despite the use of evidence-based anti-emetic guidelines. If our findings are confirmed, interventions to reduce the occurrence of CIN could be targeted toward preventing disruptions in the GBA (e.g., use of probiotics). In addition, future studies should evaluate for associations between CIN and disruptions in the oral and intestinal microbiome. Finally, future research needs to determine the relationships among other GI symptoms and their associated mechanisms and the occurrence and severity of CIN.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Table 1.**

Differences in Demographic and Clinical Characteristics Between Patients in Sample 1 With and Without CIN

Characteristic	No Nausea 36.4% (n = 130)	Nausea 63.6% (n = 227)	Statistics
	Mean (SD)	Mean (SD)	
Age (years)	58.09 (13.19)	54.90 (11.60)	t = 2.38, p = 0.018
Education (years)	16.24 (3.19)	15.88 (2.92)	t = 1.07, p = 0.285
Body mass index (kg/m <sup>2</sup> )	25.80 (4.60)	26.27 (6.20)	t = -0.82, p = 0.415
Karnofsky Performance Status score	81.97 (12.31)	74.86 (11.81)	t = 5.32, p < 0.001
Number of comorbidities	2.38 (1.39)	2.59 (1.60)	t = -1.270, p = 0.205
SCQ score	5.26 (2.90)	6.14 (3.77)	t = -2.45, p = 0.015
AUDIT score	3.18 (2.65)	2.64 (2.47)	t = 1.53, p = 0.129
Time since cancer diagnosis (years)	1.83 (3.07)	1.47 (2.90)	U, p = 0.041
Time since diagnosis (median)	0.49	0.42	
Number of prior cancer treatments	0.77 (0.42)	0.71 (0.45)	t = 1.16, p = 0.247
Number of metastatic sites including lymph node involvement	1.32 (1.30)	1.16 (1.21)	t = 1.17, p = 0.244
Number of metastatic sites excluding lymph node involvement	0.83 (1.10)	0.70 (1.04)	t = 1.08, p = 0.281
	% (n)	% (n)	
Gender			
Female	74.6 (97)	79.7 (181)	FE, p = 0.290
Male	25.4 (33)	20.3 (46)	
Ethnicity			
White	68.2 (88)	60.4 (137)	X <sup>2</sup> = 3.62, p = 0.305
Black	7.0 (9)	7.9 (18)	
Asian or Pacific Islander	16.3 (21)	16.7 (38)	
Hispanic Mixed or Other	8.5 (11)	15.0 (34)	
Married or partnered (% yes)	61.2 (79)	62.1 (139)	FE, p = 0.910
Lives alone (% yes)	22.5 (29)	23.1 (52)	FE, p = 1.000
Child care responsibilities (% yes)	16.5 (21)	24.7 (54)	FE, p = 0.080
Care of adult responsibilities (% yes)	5.1 (6)	10.0 (20)	FE, p = 0.143
Born prematurely (% yes)	3.2 (4)	6.2 (13)	FE, p = 0.306

Characteristic	No Nausea 36.4% (n = 130)	Nausea 63.6% (n = 227)	Statistics
	Mean (SD)	Mean (SD)	
Currently employed (% yes)	41.4 (53)	30.5 (69)	FE, p = 0.048
Income			
< \$30,000	12.5 (14)	27.1 (57)	U, p = 0.041
\$30,000 to < \$70,000	20.5 (23)	18.1 (38)	
\$70,000 to < \$100,000	22.3 (25)	14.8 (31)	
\$100,000	44.6 (50)	40.0 (84)	
Specific comorbidities (% yes)			
Heart disease	6.9 (9)	5.7 (13)	FE, p = 0.653
High blood pressure	35.4 (46)	30.0 (68)	FE, p = 0.291
Lung disease	6.9 (9)	11.5 (26)	FE, p = 0.197
Diabetes	10.0 (13)	11.9 (27)	FE, p = 0.728
Ulcer or stomach disease	3.8 (5)	5.7 (13)	FE, p = 0.616
Kidney disease	0.8 (1)	1.3 (3)	FE, p = 1.000
Liver disease	6.2 (8)	7.0 (16)	FE, p = 0.829
Anemia or blood disease	6.2 (8)	12.3 (28)	FE, p = 0.069
Depression	21.5 (28)	22.9 (52)	FE, p = 0.793
Osteoarthritis	10.8 (14)	12.8 (29)	FE, p = 0.616
Back pain	25.4 (33)	34.8 (79)	FE, p = 0.075
Rheumatoid arthritis	6.9 (9)	3.5 (8)	FE, p = 0.196
Exercise on a regular basis (% yes)	71.4 (90)	65.0 (141)	FE, p = 0.234
Smoking current or history of (% yes)	32.0 (41)	37.2 (83)	FE, p = 0.355
Cancer diagnosis			
Breast	41.5 (54)	38.3 (87)	X <sup>2</sup> = 4.46, p = 0.216
Gastrointestinal	31.5 (41)	37.0 (84)	
Gynecological	20.0 (26)	13.7 (31)	
Lung	6.9 (9)	11.0 (25)	
Type of prior cancer treatment			X <sup>2</sup> = 11.28, p = 0.010
No prior treatment	23.0 (29)	28.6 (63)	NS
Only surgery, CTX, or RT	39.7 (50)	45.0 (99)	NS
Surgery & CTX, or Surgery & RT, or CTX & RT	27.0 (34)	12.7 (28)	0 > 1
Surgery & CTX & RT	10.3 (13)	13.6 (30)	NS
CTX cycle length			X <sup>2</sup> = 8.23, p = 0.016
14 day cycle	39.2 (51)	53.7 (122)	0 < 1
21 day cycle	53.8 (70)	38.3 (87)	0 > 1
28 day cycle	6.9 (9)	7.9 (18)	NS
Emetogenicity of CTX			X <sup>2</sup> = 2.17, p = 0.337

Characteristic	No Nausea 36.4% (n = 130)	Nausea 63.6% (n = 227)	Statistics
	Mean (SD)	Mean (SD)	
Minimal/Low	13.8 (18)	15.0 (34)	
Moderate	68.5 (89)	61.2 (139)	
High	17.7 (23)	23.8 (54)	
Antiemetic regimens			$X^2 = 8.06, p = 0.045$
None	7.7 (10)	4.4 (10)	NS
Steroid alone or serotonin receptor antagonist alone	21.5 (28)	14.5 (33)	NS
Serotonin receptor antagonist and steroid	49.2 (64)	47.6 (108)	NS
NK-1 receptor antagonist and two other antiemetics	21.5 (28)	33.5 (76)	NS

Abbreviations: AUDIT = Alcohol Use Disorders Identification Test, CIN = chemotherapy-induced nausea, CTX = chemotherapy, FE = Fisher's Exact test, kg = kilograms, m<sup>2</sup> = meter squared, NK-1 = Neurokinin-1, NS = not significant, RT = radiation therapy, SCQ = Self-administered Comorbidity Questionnaire, SD = standard deviation, U = Mann-Whitney U test, X<sup>2</sup> = Chi square



**Table 2.**

Differences in Demographic and Clinical Characteristics Between Patients in Sample 2 With and Without CIN

Characteristic	No Nausea 51.1% (n = 180)	Nausea 48.9% (n = 172)	Statistics
	Mean (SD)	Mean (SD)	
Age (years)	57.80 (12.10)	55.53 (11.37)	t = 1.81, p = 0.071
Education (years)	16.82 (2.83)	15.90 (2.97)	t = 2.95, p = 0.003
Body mass index (kg/m <sup>2</sup> )	26.54 (5.86)	26.82 (6.31)	t = -0.44, p = 0.662
Karnofsky Performance Status score	82.44 (11.03)	76.80 (12.22)	t = 4.33, p < 0.001
Number of comorbidities	2.40 (1.36)	2.55 (1.46)	t = -1.01, p = 0.312
SCQ score	5.38 (2.81)	5.92 (3.22)	t = -1.69, p = 0.091
AUDIT score	2.96 (2.50)	3.09 (3.03)	t = -0.35, p = 0.728
Time since cancer diagnosis (years)	2.18 (3.66)	2.27 (3.86)	U, p = 0.461
Time since diagnosis (median)	0.44	0.45	
Number of prior cancer treatments	1.80 (1.58)	1.81 (1.62)	t = -0.08, p = 0.940
Number of metastatic sites including lymph node involvement	1.36 (1.28)	1.18 (1.30)	t = 1.31, p = 0.190
Number of metastatic sites excluding lymph node involvement	0.92 (1.12)	0.73 (1.14)	t = 1.58, p = 0.115
	% (n)	% (n)	
Gender			
Female	79.4 (143)	82.0 (141)	FE, p = 0.590
Male	20.6 (37)	18.0 (31)	
Ethnicity			X <sup>2</sup> = 10.09, p = 0.018
White	77.0 (134)	63.1 (106)	0 > 1
Black	3.4 (6)	9.5 (16)	NS
Asian or Pacific Islander	9.8 (17)	16.1 (27)	NS
Hispanic Mixed or Other	9.8 (17)	11.3 (19)	NS
Married or partnered (% yes)	69.4 (125)	62.9 (107)	FE, p = 0.214
Lives alone (% yes)	16.9 (30)	22.8 (39)	FE, p = 0.180
Child care responsibilities (% yes)	19.0 (34)	29.8 (51)	FE, p = 0.024
Care of adult responsibilities (% yes)	7.7 (13)	11.0 (17)	FE, p = 0.342
Born prematurely (% yes)	2.9 (5)	7.3 (12)	FE, p = 0.083

Characteristic	No Nausea 51.1% (n = 180)	Nausea 48.9% (n = 172)	Statistics
	Mean (SD)	Mean (SD)	
Currently employed (% yes)	33.0 (59)	35.7 (61)	FE, p = 0.653
Income			
< \$30,000	15.5 (25)	27.6 (43)	U, p = 0.001
\$30,000 to < \$70,000	19.3 (31)	21.8 (34)	
\$70,000 to < \$100,000	13.7 (22)	15.4 (24)	
\$100,000	51.6 (83)	35.3 (55)	
Specific comorbidities (% yes)			
Heart disease	8.3 (15)	2.9 (5)	FE, p = 0.037
High blood pressure	30.0 (54)	29.1 (50)	FE, p = 0.907
Lung disease	13.9 (25)	8.1 (14)	FE, p = 0.092
Diabetes	5.6 (10)	10.5 (18)	FE, p = 0.115
Ulcer or stomach disease	3.3 (6)	6.4 (11)	FE, p = 0.218
Kidney disease	0.6 (1)	1.7 (3)	FE, p = 0.362
Liver disease	7.2 (13)	6.4 (11)	FE, p = 0.834
Anemia or blood disease	9.4 (17)	18.6 (32)	FE, p = 0.014
Depression	17.2 (31)	28.5 (49)	FE, p = 0.015
Osteoarthritis	13.9 (25)	13.4 (23)	FE, p = 1.000
Back pain	25.6 (46)	27.9 (48)	FE, p = 0.632
Rheumatoid arthritis	5.6 (10)	2.3 (4)	FE, p = 0.172
Exercise on a regular basis (% yes)	69.8 (125)	70.8 (121)	FE, p = 0.907
Smoking current or history of (% yes)	39.5 (70)	33.1 (56)	FE, p = 0.221
Cancer diagnosis			X <sup>2</sup> = 12.15, p = 0.007
Breast	34.4 (62)	43.0 (74)	NS
Gastrointestinal	20.6 (37)	29.7 (51)	NS
Gynecological	28.3 (51)	17.4 (30)	NS
Lung	16.7 (30)	9.9 (17)	NS
Type of prior cancer treatment			X <sup>2</sup> = 1.38, p = 0.711
No prior treatment	17.9 (32)	19.9 (34)	
Only surgery, CTX, or RT	46.4 (83)	41.5 (71)	
Surgery & CTX, or Surgery & RT, or CTX & RT	21.2 (38)	20.5 (35)	
Surgery & CTX & RT	14.5 (26)	18.1 (31)	
CTX cycle length			X <sup>2</sup> = 10.30, p = 0.006
14 day cycle	26.7 (48)	42.4 (73)	0 < 1
21 day cycle	66.1 (119)	50.0 (86)	0 > 1
28 day cycle	7.2 (13)	7.6 (13)	NS
Emetogenicity of CTX			X <sup>2</sup> = 8.05, p = 0.018

Characteristic	No Nausea 51.1% (n = 180)	Nausea 48.9% (n = 172)	Statistics
	Mean (SD)	Mean (SD)	
Minimal/Low	27.2 (49)	18.0 (31)	NS
Moderate	59.4 (107)	58.7 (101)	NS
High	13.3 (24)	23.3 (40)	0 < 1
Antiemetic regimens			X <sup>2</sup> = 15.65, p = 0.001
None	11.7 (20)	8.4 (14)	NS
Steroid alone or serotonin receptor antagonist alone	30.4 (52)	15.0 (25)	0 > 1
Serotonin receptor antagonist and steroid	41.5 (71)	49.1 (82)	NS
NK-1 receptor antagonist and two other antiemetics	16.4 (28)	27.5 (46)	NS

Abbreviations: AUDIT = Alcohol Use Disorders Identification Test, CIN = chemotherapy-induced nausea, CTX = chemotherapy, FE = Fisher's Exact test, kg = kilograms, m<sup>2</sup> = meter squared, NK-1 = Neurokinin-1, NS = not significant, RT = radiation therapy, SCQ = Self-administered Comorbidity Questionnaire, SD = standard deviation, U = Mann-Whitney U test, X<sup>2</sup> = Chi square

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**Table 3.****Multiple Logistic Regression Analyses Predicting Nausea Group Membership**

Sample 1 (n = 334)			
Predictor	Odds Ratio	95% CI	p-value
Karnofsky Performance Status score	0.95	0.93, 0.97	< 0.001
CTX cycle length			0.023
21 day cycle vs 14 day cycle	0.50	0.31, 0.83	0.007
28 day cycle vs 14 day cycle	0.87	0.34, 2.27	0.780
21 day cycle vs 28 day cycle	0.58	0.22, 1.50	0.256
Type of prior cancer treatment			0.031
Only surgery, CTX, or RT vs No prior treatment	0.95	0.53, 1.71	0.860
Surgery & CTX, or Surgery & RT, or CTX & RT vs No prior treatment	0.38	0.19, 0.78	0.009
Surgery & CTX & RT vs No prior treatment	0.93	0.39, 2.18	0.861
Surgery & CTX, or Surgery & RT, or CTX & RT vs Only surgery, CTX, or RT	0.40	0.21, 0.78	0.007
Surgery & CTX & RT vs Only surgery, CTX, or RT	0.98	0.43, 2.20	0.955
Surgery & CTX, or Surgery & RT, or CTX & RT vs Surgery & CTX & RT	0.41	0.17, 1.00	0.050
Overall model fit: df = 6, $X^2 = 43.46$ , $p < 0.001$			
Sample 2 (n = 294)			
Predictor	Odds Ratio	95% CI	p-value
Child care responsibilities	1.90	1.05, 3.42	0.033
Karnofsky Performance Status score	0.96	0.94, 0.98	< 0.001
Emetogenicity of CTX			0.016
Moderate vs Minimal/Low	1.60	0.82, 3.11	0.166
High vs Minimal/Low	3.40	1.47, 7.85	0.004
Moderate vs High	0.47	0.23, 0.97	0.041
Cancer diagnosis			0.003
Gastrointestinal cancer vs Breast cancer	1.76	0.90, 3.46	0.099
Gynecological cancer vs Breast cancer	0.64	0.32, 1.28	0.207
Lung cancer vs Breast cancer	0.35	0.15, 0.84	0.019
Gastrointestinal cancer vs Lung cancer	5.00	1.94, 12.91	0.001
Gynecological cancer vs Lung cancer	1.81	0.70, 4.71	0.225
Gynecological cancer vs Gastrointestinal cancer	0.36	0.18, 0.75	0.006
Overall model fit: df = 7, $X^2 = 48.34$ , $p < 0.001$			

Abbreviations: CI = confidence interval, CTX = chemotherapy, RT = radiotherapy

**Table 4 -**

Perturbed Gut-Brain Axis Related KEGG Pathways Between Oncology Patients With and Without Chemotherapy-Induced Nausea

Pathway ID	Pathway Name	pGlobal.FWER
Mucosal inflammation		
hsa04060	Cytokine-cytokine receptor interaction	0.00084
hsa04010	Mitogen activated protein kinase signaling pathway	0.00306
hsa04064	Nuclear factor $\kappa$ B signaling pathway *	0.00982
hsa04062	Chemokine signaling pathway	0.00084
Disruption of gut microbiome		
hsa04672	Intestinal immune network for immunoglobulin A production	0.00917
hsa04064	Nuclear factor $\kappa$ B signaling pathway *	0.00982
hsa03320	Peroxisome-proliferation-activated receptor signaling pathway	0.00084
hsa04659	Interleukin-17 producing helper T cells differentiation pathway	0.00516
hsa04530	Tight junction	0.00084
hsa04612	Antigen processing and presentation	0.00652

\* Perturbed pathway associated with more than one mechanism

Abbreviation: KEGG = Kyoto Encyclopedia of Genes and Genomes, FWER = family-wise error rate