Title

Identification of Demographic and Clinical Characteristics, Differentially Expressed Genes, and Differentially Perturbed Pathways Associated with Chemotherapy-Induced Nausea

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Identification of Demographic and Clinical Characteristics, Differentially Expressed Genes, and Differentially Perturbed Pathways Associated with Chemotherapy-Induced Nausea

by

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DISSERTATION

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- Singh KP, Kober KM, Dhruva AA, Flowers E, Paul SM, Hammer MJ, Cartwright F, Wright F, Conley YP, Levine JD, Miaskowski C. Risk Factors Associated With Chemotherapy-Induced Nausea in the Week Prior to the Next Cycle and Impact of Nausea on Quality of Life Outcomes. Journal of Pain and Symptom Management. 2018. doi: 10.1016/j.jpainsymman.2018.05.019.

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Abstract

Identification of Demographic and Clinical Characteristics, Differentially Expressed Genes, and Differentially Perturbed Pathways Associated with Chemotherapy-Induced Nausea

Komal Preet Singh

Despite advancements in antiemetic prophylaxis, chemotherapy-induced nausea (CIN) continues to be a significant clinical problem. Between 30% to 60% of oncology patients experience CIN. While a number of demographic and clinical characteristics are established risk factors CIN, these phenotype risk factors do not explain all of the variance in the occurrence of CIN. The purposes of this dissertation research were to: perform a systematic review of the literature on the associations between single nucleotide polymorphisms (SNPs) in candidate genes and the occurrence of CIN; determine additional risk factors associated with the occurrence of CIN; and determine additional molecular mechanisms associated with the occurrence of CIN.

Sixteen studies evaluated for associations between genomic markers and the occurrence and/or severity of chemotherapy-induced nausea and vomiting (CINV). Candidate genes in the major mechanistic pathways for CINV (i.e., serotonin receptor pathway, drug transport pathway and/or drug metabolism) were evaluated for associations with the occurrence and severity of CINV. In brief, none of the SNPs in these mechanistic pathways were associated with CIN occurrence.

Demographic and clinical risk factors were evaluated for their associations with CIN occurrence. In addition, the impact of concurrent symptoms, stress associated with cancer and its treatment, as well as quality of life (QOL) outcomes on the occurrence of CIN were investigated in patients prior to their next dose of chemotherapy (CTX). Modifiable risk factors identified in this study include: having child-care responsibilities; poorer functional status; and higher levels

of depression, sleep disturbance, evening fatigue, perceived stress, and intrusive thoughts and feelings. Patients who reported CIN experienced decrements in QOL outcomes.

Because findings regarding associations between mechanistically-based candidate genes and CIN occurrence were inconclusive, a hypothesis-generating study was undertaken to uncover novel mechanisms associated with CIN occurrence. Findings from this dissertation research suggest that a number of differentially expressed genes and perturbed pathways in the gut-brain axis are associated with the occurrence of CIN. CTX-induced changes in the GBA that may contribute to the occurrence of CIN include: mucosal inflammation and disruption of the gut microbiome. This dissertation concludes with implications for clinical practice and directions for future research.

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Chapter 1:

Introduction to Dissertation

Chemotherapy-induced nausea (CIN) is a common side effect of cancer chemotherapy (CTX). CIN occurs in 30% to 60% of oncology patients receiving CTX.(1-4) If not controlled, CIN can lead to dehydration,(5) weight loss,(5) decline in quality of life (QOL),(6, 7) and in some cases discontinuation of cancer treatment.(8) While the prevention and treatment of chemotherapy-induced vomiting (CIV) is well managed with the advent of antiemetic prophylaxis, unrelieved CIN remains a significant clinical problem.(9) At the initiation of CTX, patients consistently list nausea as one of their greatest fears.(10, 11) Nausea is an unpleasant sensation experienced in the back of the throat and epigastrum that may or may not result in the expulsion of stomach contents.(12)

In terms of predictors of CIN, females and younger patients with higher trait anxiety and a history of nausea are at highest risk.(13-19) The intrinsic emetogenic potential of the CTX is an important contributing factor for CIN.(20-23) The emetogenicity of a CTX regimen can be categorized into one of four emetic risk groups, namely: high (>90%), moderate (30-90%), low (10-30%), and minimal (<10%). These percentages reflect the percentage of patients who will experience chemotherapy-induced nausea and vomiting (CINV) if they receive a particular CTX regimen without any prophylaxis.(24, 25) Despite administration of antiemetic prophylaxis based on this emetogenicity schema, patients continue to experience CIN. Of note, patients with a history of high alcohol intake are at a lower risk for CIN.(15, 16)

Types of CIN

Depending on the timing of its occurrence, CIN is categorized as acute, delayed, anticipatory,(26) breakthrough, or refractory.(9, 27) Acute CIN occurs within the first 24 hours

and its maximum intensity occurs 5 to 6 hours after CTX administration. Delayed CIN occurs 24 hours after CTX administration, reaches peak intensity between 48 and 72 hours after CTX administration, and can persist for 5 to 7 days. Delayed CIN is more common in people who experience acute CIN. In a multinational study of patients receiving moderately and highly emetogenic CTX,(6) 36.2% reported acute and 54.3% reported delayed CIN.

Anticipatory CIN usually occurs prior to the actual administration of CTX and is based on previous experiences and expectancies about the occurrence of nausea.(28-30) The incidence rate for anticipatory CIN ranges from 18% to 57%.(9) Anticipatory CIN can be triggered by certain odors, tastes, thoughts, or even anxiety related to treatment. Anticipatory CIN is more difficult to control than acute or delayed CIN.(31) Pre-CTX anticipatory CIN is a significant predictor of a future episode of CIN. Of the patients who experience pre-CTX anticipatory CIN, only 30% achieve a complete response during their first CTX cycle.(32) In one study,(33) 8% to 14% of patients reported anticipatory CIN that increased in frequency and intensity over each subsequent cycle.

Breakthrough CIN occurs within five days after CTX administration even when guideline directed prophylactic antiemetic agents are given to control nausea. The occurrence rates for breakthrough CIN range from 22%(34) to 40%.(27) Refractory CIN occurs in subsequent CTX cycles when guideline directed prophylactic antiemetic agents fail to control nausea during previous cycles.

Compared to anticipatory, breakthrough, and refractory CIN, the mechanisms involved in acute and delayed CIN are better understood. Acute and delayed CIN are considered to be complex, multifactorial processes that involve several anatomic sites and neurotransmitter pathways.(35) The most well studied pathway that leads to acute and delayed CIN is the

serotonin receptor pathway. While some anatomic pathways for acute and delayed CIN overlap, other pathways are distinct.(36, 37)

Mechanisms for CIN

In terms of the mechanisms that underlie CIN, acute CIN occurs when CTX administration generates free radicals that damage the enterochromaffin cells lining the gastrointestinal (GI) mucosa of the stomach.(25) Free radicals stimulate enterochromaffin cells to release excessive amounts of 5-hydroxytryptamine (5-HT), also known as serotonin, that binds to 5-HT_3 receptors on vagal afferents.(38) This binding activates vagal afferents to release Substance-P (SP) that binds to the tachykinin receptor Neurokinin-1 (NK-1) and increases the activity of the vagal afferents.(39, 40) Vagal afferents innervate the bowel and mediate the autonomic signaling between the GI tract and the brain. Vagal afferent fibers innervate both the enteric nervous system (ENS) and the medulla. Vagal afferents terminate in the medial nucleus of the solitary tract (NTS) and the dorsal vagal complex (DVC) in the medulla.(39) Activation of the NTS and vagal efferents by vagal afferents leads to the sensation of nausea.(39)

In terms of the mechanism for delayed CIN, the chemoreceptor trigger zone (CTZ) is activated by emetogenic signals that cross the blood-brain-barrier (BBB). The CTZ is exposed to circulating blood and CTX can cross the BBB in this region.(25) The 5-HT and SP that are released during acute CIN may cross the BBB to augment the process of delayed CIN. Neurons in the CTZ activate the NTS and neurons from the NTS project the signal to the central pattern generator. Activation of the central pattern generator and vagal efferents lead to delayed CIN.(39) Patients who experience acute CIN are more likely to experience delayed CIN.(41, 42)

In addition to the serotonin receptor pathway, the drug metabolism pathway and the drug transport pathway have been investigated for their associations with CIN occurrence. These pathways influence the turnover rates of CTX and antiemetic drugs as well as their intracellular

transport in the GI tract and the BBB. Drug metabolizing proteins belong to a family of cytochrome P450 isoenzymes that bio-transform drugs through oxidation. Of the cytochrome P450 isoenzymes, the cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*) gene is well studied for its associations with CIN occurrence. ATP binding cassette proteins are transmembrane proteins present on the membranes of cells that line the BBB, as well as on the cell membranes that line the GI tract. Their primary function is intracellular drug transport. ATP binding cassette subfamily B member 1 (ABCB1) is involved in the transport of CTX in the GI tract as well as in the central nervous system. *ABCB1* is well studied for its association with CIN occurrence.

Guideline directed treatment

The intrinsic emetogenicity of a CTX regimen became the standard for the development of evidence-based guidelines for antiemetic treatment of CINV.(24) Based on National Comprehensive Cancer Network (NCCN) guidelines, to prevent the occurrence of acute and delayed emesis, a combination of a 5-HT_3 antagonist, a NK-1 antagonist, and dexamethasone should be given prior to the administration of a moderate or a high emetic risk intravenous CTX regimen. Alternatively, an olanzapine containing regimen can be given. A dopamine antagonist, a 5-HT_3 antagonist, or dexamethasone is recommended before the administration of a low emetic risk intravenous CTX. No routine prophylaxis is recommended before minimal emetic risk CTX administration.(9)

Focus of this dissertation research

Inter-individual differences in phenotypic and molecular characteristics, identified to date, do not explain all the variance in the occurrence of CIN. Therefore, the purposes of this dissertation study were to: perform a systematic review of the literature on the associations between single nucleotide polymorphisms (SNPs) in candidate genes and the occurrence of CIN;

additional phenotypic risk factors associated with the occurrence of CIN; and determine additional mechanisms that may be associated with occurrence of CIN in oncology patients receiving CTX. This dissertation consists of three papers. The first paper is a systematic review of the literature on occurrence and severity of CINV.(43) The second paper reports on phenotypic risk factors associated with CIN and the impact of nausea on quality of life (QOL) outcomes of oncology patients receiving CTX.(44) The third paper reports on associations between the occurrence of CIN and differentially expressed genes and perturbed pathways in gut-brain.

The first paper reports on findings from a systematic review of sixteen studies on associations between single nucleotide polymorphisms (SNPs) in candidate genes and the occurrence of CIN, as well as on associations between antiemetic efficacy and SNPs in a number of candidate genes. SNPs in various 5-HT receptors were well studied for associations with the occurrence of CIN. Across six studies that evaluated 22 SNPs in the serotonin receptor pathway,(45-50) only one found an association with CIN severity.(50) Across six studies,(51-56) that evaluated seven SNPs and one haplotype in the drug transport pathway, five found associations with CIN occurrence.(52-56) Across three studies, that evaluated three SNPs and an ultra-metabolizer polymorphism with more than two active copies of the gene as a result of duplication in *CYP2D6*,(48, 54, 57) one found an association with CIN severity.(48)

Across twelve studies that evaluated for associations between antiemetic efficacy and SNPs as well as haplotypes in a number of candidate genes, (45-49, 51, 52, 54-58) three studies found associations between antiemetic efficacy and two SNPs and one haplotype in serotonin receptor genes;(45-47) five studies found associations between drug transport pathway genes and antiemetic efficacy;(51, 52, 54, 56, 58) and two studies found associations between drug metabolizing pathway genes and antiemetic efficacy.(48, 57) None of the SNPs in the serotonin

receptor gene (45, 46) and none of the alleles of the cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*) gene (54) were associated with CIN occurrence. Three SNPs and two haplotypes in the *ABCB1* gene (52-56) showed inconsistent findings regarding an association with CIN occurrence. This chapter is a reprint of a manuscript that is published in *Critical Reviews in Hematology and Oncology*.(43)

In the second paper, we evaluated for the occurrence, severity, and distress of CIN and evaluated for differences in demographic and clinical characteristics, symptom severity, perceived stress, and QOL outcomes between patients who did and did not report CIN in the week prior to their next dose of CTX. In addition, we determined which demographic, clinical, symptom, and stress characteristics were associated with the occurrence of nausea. Approximately 48% of oncology patients in our study reported nausea in the week prior to their next cycle of CTX. In our multivariate model, the phenotype characteristics that were associated with CIN group membership included: less education; having child care responsibilities; poorer functional status; higher levels of depression, sleep disturbance, evening fatigue, and intrusive thoughts; as well as receipt of CTX on a 14-day CTX cycle and receipt of an antiemetic regimen that contained a serotonin receptor antagonist and a steroid. Patients in the CIN group experienced clinically meaningful decrements in QOL. While CIN negatively impacted patients' QOL, the identification of new phenotypic risk factors in our study (e.g., poorer functional status, higher levels of stress) may help identify patients at risk for developing CIN and determine appropriate preemptive interventions for these patients. This chapter is a reprint of a manuscript published in the *Journal of Pain and Symptom Management*.(44)

In the third paper, we evaluated for differentially expressed genes and perturbed pathways associated with the gut-brain axis (GBA) across the two independent samples of patients with and without CIN, after controlling for significant demographic and clinical

characteristics. Occurrence of CIN was assessed using the Memorial Symptom Assessment Scale. Gene expression analyses was performed in two independent samples (i.e., sample 1, n = 357 and sample 2, n = 352) using ribonucleic-acid-sequencing (RNA-seq) and microarray gene expression methodologies. Fisher's Combined Probability test was used to combine the differential gene expression tests from both datasets and to determine the overall number of significantly perturbed pathways between the two CIN groups. CIN occurrence was reported by 227 (63.6%) of patients in sample 1 and 172 (48.9%) patients in sample 2. A number of differentially expressed genes and perturbed pathways associated with the GBA were found in patients with CIN. Our findings suggest that CTX-induced changes in the GBA occur through mucosal inflammation and disruption of gut microbiome.

Taken together, our finding suggest that a number of demographic and clinical characteristics, as well as symptoms and intrusive thoughts are risk factors associated with the occurrence of CIN. Patients who experience CIN experience poorer QOL outcomes. Based on our findings related to differential gene expression and pathway perturbations, CTX-induced changes in mucosal integrity and alterations in the gut microbiome may contribute to the occurrence of CIN in the week prior to the patients' next dose of CTX.

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Chapter 2

A review of the literature on the relationships between genetic polymorphisms and chemotherapy-induced nausea and vomiting

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Abstract

Despite current advances in antiemetic treatments, between 30% to 60% of oncology patients experience chemotherapy-induced nausea (CIN) and 13% to 33% report chemotherapy-induced vomiting (CIV). Inter-individual differences are observed in the occurrence and severity of chemotherapy-induced nausea and vomiting (CINV). This review summarizes and critiques studies on associations between occurrence and severity of CINV and polymorphisms in serotonin receptor, drug metabolism, and drug transport pathway genes. Sixteen studies evaluated the associations between the occurrence and/or severity of CINV and single nucleotide polymorphisms (SNPs). Across these studies, three SNPs in 5-hydroxytryptamine receptor (*5-HT3R*) genes, two alleles of the cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*) gene, and three SNPs in ATP binding cassette subfamily B member 1 (*ABCB1*) gene were associated with the occurrence and severity of CINV. Given the limited number of polymorphisms evaluated, additional research is warranted to identify new mechanisms to develop more targeted therapies.

Keywords: nausea; vomiting; serotonin; drug metabolism; drug transport; antiemetics

INTRODUCTION

Despite current advances in antiemetic treatments, between 30% to 60% of oncology patients experience chemotherapy-induced nausea (CIN) and 13.3% to 32.5% report chemotherapy-induced vomiting (CIV).(1-3) Despite the use of guideline directed antiemetic regimens, CIN continues to be one of the most severe side effects of chemotherapy (CTX).(4) Inter-individual differences are observed in the occurrence and severity of chemotherapy-induced nausea and vomiting (CINV). Phenotypic characteristics associated with increased risk of CINV include: age under 50 years, female gender, higher trait anxiety, a history of motion sickness, a history of morning sickness, decreased alcohol intake, dehydration, malnutrition, recent surgery, and radiation therapy.(5-8)

Treatment characteristics associated with increased risk for CINV include: higher pretreatment expectations for CINV; susceptibility to conditioned responses triggered by odors and tastes in the oncology clinic; occurrence of CINV during a previous CTX treatment; and feelings of warmth, dizziness, or lightheadedness after CTX.(9, 10) In addition, the intrinsic emetogenic potential of the CTX is an important factor that contributes to the occurrence and severity of CINV.(11-14) Finally, non-adherence with the antiemetic treatment regimen during the CTX cycle increases the risk for CINV.(8)

While these phenotypic characteristics help to identify high risk patients, they do not explain all of the inter-individual variability in the occurrence and severity of CINV. For example, in a study of risk factors for antiemetic failure,(15) 46% of the patients with three risk factors (i.e., female gender, younger age, no history of alcohol use) and 9% of the patients with no risk factors experienced antiemetic treatment failure. Recent evidence suggests that polymorphisms in genes involved in the nausea and vomiting pathways may influence oncology patients' risk for CINV and/or their responses to antiemetics. To date, four reviews have

summarized findings from studies on associations between antiemetic efficacy and genetic polymorphisms in oncology patients receiving CTX.(16-19)

In the first review,(17) findings from six pharmacogenetic studies of antiemetic efficacy were summarized. The specific genes evaluated across these six studies were: 5-hydroxytryptamine 3A receptor (*HTR3A*), *HTR3B*, *HTR3C*, ATP binding cassette subfamily B member 1 (*ABCB1*), and cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*). The second review focused on an evaluation of differences in the efficacy of 5HT3 receptor antagonists associated with a number of genetic polymorphisms.(16) While focused on a single mechanism, this review extended the findings from the previous review(17) with a summary of four additional studies. The third review focused on the phamacogenetics of CINV.(18) This 2015 review was organized using the major mechanisms that contribute to antiemetic efficacy. Across nine studies, seven of which were highlighted in the previous reviews,(16, 17) associations between antiemetic efficacy and polymorphisms in *HTR3B*, *HT3RC*, *HT3RD*, *neurokinin-1 (NK-1*) *receptor*, *ABCB1*, organic cation transporter protein *(OCT1*), and *CYP2D6* genes were described.

In the fourth narrative review that focused on the nursing implications of the pharmacogenomic studies of antiemetic efficacy,(19) only one additional study was summarized. The major focus of all four papers was to summarize the pharmacogenomic findings within the context of the major mechanisms that are targeted by antiemetics to decrease CINV, namely: 5HT3, drug transport, and drug metabolism pathways.

However, none of these papers provided a comprehensive synthesis of these studies that included a detailed description of the associations between genetic polymorphisms and the occurrence and severity of CINV; a critique of the studies' designs and the methods used to assess CINV; a description of study limitations; and directions for future research. Therefore, the

purposes of this review of the relationships between genetic polymorphisms and CINV are to: 1) describe salient study characteristics; 2) summarize and critique the instruments used to assess CINV and the timing of the assessments; 3) synthesize findings on associations between the occurrence and severity of CINV and genetic polymorphisms; and 4) synthesize findings on associations between antiemetic efficacy and genetic polymorphisms.

METHODS

Literature search

A systematic electronic literature search was conducted using three databases: PubMed®, Excerpta Medica Database (EMBASE®), and the Cumulative Index to Nursing and Allied Health Literature (CINAHL®). A combination of keywords used to identify relevant studies were: *chemotherapy-induced nausea and vomiting* or *chemotherapy-induced vomiting* or *chemotherapy-induced nausea* AND *gene* or *genetics* or *polymorphisms* or *gene expression* or *candidate genes*. Studies were included if they met the following criteria: (1) the entire sample had a cancer diagnosis; (2) oncology patients were assessed for CIN and/or CIV; (3) oncology patients were genotyped; and (4) associations between the occurrence and/or severity of CIN and/or CIV, with or without antiemetic drugs, and patient genotype were described. An additional inclusion criterion was that the studies were published in English between 2000 and 2016 because the human genome was sequenced in 2000. Studies were excluded: (1) if the timing of the CIN or CIV assessments was not reported; (2) if they evaluated postoperative nausea and vomiting or radiotherapy-induced nausea and vomiting; and (3) if genotype associations were evaluated only in the context of the pharmacokinetics of the CTX.

As shown in Figure 1, the search strategy yielded 202 studies in PubMed®, 476 studies in EMBASE®, and 12 studies in CINAHL®. A total of 623 studies were excluded because the majority of these studies did not evaluate CINV. Of the 51 studies that did evaluate CINV, 35

were excluded because: 11 did not report the timing of the CIN or CIV assessment; 4 evaluated postoperative nausea and vomiting or radiotherapy-induced nausea and vomiting; 5 did not have genotype data; 1 evaluated genetic associations in the context of CTX pharmacokinetics; and 14 were review articles.

These review articles had the following foci: one was on associations between postoperative nausea and vomiting and genetic polymorphisms; five focused on protein structure of receptors involved in CINV; four described the pathophysiology of CINV and pharmacological interventions; and the four summarized above,(16-19) described associations between antiemetic efficacy and genetic polymorphisms. Duplicate articles across the databases were removed and screened based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) criteria.(20) Based on our pre-specified inclusion criteria, sixteen studies are included in this review.(21-36)

Data synthesis

These sixteen studies were summarized using the following prespecified evaluation criteria: author, year, purpose, and study design; emetogenicity of the CTX regimen; major study outcome(s); gene(s) and associated polymorphism(s) classified by function; sample characteristics (i.e., sample size, age, gender, diagnosis, treatment setting, antiemetic treatment); assessment of CINV (i.e., instrument(s), timing of CINV assessments); genotyping methods; statistical analyses; major findings; strengths; and limitations (Supplementary Table 1). Given the heterogeneity of the descriptive data among the studies in terms of sample characteristics, assessment of CINV, timing of CINV assessments, types of genotyping methods, specific polymorphisms evaluated, and the types of CTX, the results are summarized in tabular and narrative form.

RESULTS

Sample and treatment characteristics

Study characteristics – All sixteen studies used a prospective cohort design. While all sixteen studies recruited patients from the outpatient setting, four included hospitalized patients.(21-23, 28) Six studies were conducted in Germany,(21-23, 25, 27, 29) two in the United States,(33, 34) two in Turkey,(24, 36) and one each in China,(32) Japan,(31) Indonesia,(28) Israel,(35) Australia,(26) and Spain.(30)

Patient characteristics – Sample sizes ranged from 64(31) to 2,886(34) patients. Six had less than 200 patients.(25-27, 31, 33, 35) Across twelve studies that reported patients' age,(21-25, 28-32, 35, 36) the weighted grand mean age was 54.8 years. Of the remaining four studies, one did not report the patients' age (26) and three reported an age range,(27) a median age,(33) or both(34). Across fourteen studies, the weighted grand mean percentage of female patients was 51.1%. Two studies did not report the patients' gender distribution.(26, 29) When the study with 2,886 patients was removed,(34) the grand mean percentage of females was 64.3%.

Across the 16 studies, various cancer diagnosis were included (e.g., breast cancer, lung cancer, non-small cell lung cancer, lymphoma, myeloma, ovarian cancer, nasopharyngeal cancer, vulvar cancer, cervical cancer, colorectal cancer, gastrointestinal cancer, genitourinary cancer). In six studies,(21-24, 29, 36) between 27.6% and 63.0% of the patients had breast cancer. In four studies,(25, 27, 31, 35) 100% of the patients had breast cancer. In two studies,(30, 34) 100% of the patients had stage III or higher colon cancer. In one study,(32) all of the patients had acute myeloid leukemia. In another study,(33) all of the patients had non-small cell lung cancer. One study did not report the patients' cancer diagnoses.(26)

Types of CTX – In nine studies,(21-25, 27, 31, 35, 36) across a total of 1657 patients, 865 received cyclophosphamide alone or a combination CTX regimen that included

cyclophosphamide. In seven studies,(21-24, 28, 33, 36) across a total of 1501 patients, 615 received a platinum-based CTX treatment. In two studies,(30, 34) 3903 patients received 5-flurouracil (5-FU) or a 5-FU based CTX regimen (e.g., a combination of folinic acid, 5-FU, and oxaliplatin (FOLFOX); a combination of folinic acid and 5-FU (FOLFIRI)). In one study of 216 patients,(24) 161 received an anthracycline-based CTX regimen. In another study,(32) all 215 patients received cytarabine.

Emetogenicity of CTX regimens – Of the fourteen studies with available data, the CTX regimens were of moderate to high emetogenicity based on the classification scheme proposed by Hesketh and colleagues.(37, 38) One study did not report on the emetogenicity of the CTX regimen.(26) One did not report the CTX regimen administered.(29)

Antiemetic treatment – Four studies did not report the specific antiemetic regimen administered.(30, 33-35) In twelve studies,(21-29, 31, 32, 36) patients received serotonin antagonists prophylactically. In terms of the specific drugs, in ten studies, patients received a standardized regimen of tropisetron and/or ondansetron.(21-29, 32) In the remaining studies, patients received granisetron,(31, 36), dolasetron,(26) or metoclopramide(28) for delayed CINV. In five studies,(25, 27, 28, 31, 36) dexamethasone was given with a standardized regimen that contained a serotonin antagonist.

Methods used to assess CIN and CIV

Assessment of CIN occurrence – The occurrence of CIN was evaluated in nine studies.(25, 27, 28, 30-34, 36) In three studies,(25, 27, 31) a patient diary was used to assess CIN occurrence. In two of these studies,(25, 27) patients documented the occurrence of CIN on an hourly basis for two days after the first cycle of CTX. In the third study,(31) daily assessments of CIN were done for 5 days following CTX administration.

Four studies(28, 32-34) used the National Cancer Institute Common Toxicity Criteria (NCICTC) to assess CIN occurrence. Three studies(28, 32, 34) used NCICTC version 3 and one study(33) used NCICTC version 4. In two studies,(28, 32) the occurrence of acute CIN was categorized as absent (i.e., grades 1 or 2) or present (i.e., grades 3 or 4). In the same two studies,(28, 32) the occurrence of CIN was assessed using a visual analog scale (VAS) that ranged from 0 mm to 100 mm. CIN occurrence was categorized as absent (i.e., a score of <5 mm on the VAS) or present (i.e., a score of >5 mm on the VAS). In another study that used NCICTC version 3,(34) patients were assessed biweekly for the occurrence of CIN, which was categorized as absent (i.e., grades 1 or 2) or present (i.e., grades 3 or 4).

In one study that used NCICTC version 4,(33) CIN occurrence was self-reported at the oncology clinic prior to CTX administration and before each subsequent cycle and was categorized as absent (i.e., grades 1 or 2) or present (i.e., grade 3). Other instruments used to assess the occurrence of CIN included the World Health Organization (WHO) toxicity grading scale(30, 39) and a daily questionnaire that rated the severity of CIN as none, slight, moderate, or severe(36). In the study that used the WHO toxicity grading scale,(30) the timing of the CIN assessments was not reported. The occurrence of CIN was categorized as absent (i.e., WHO grades 1 or 2) or present (i.e., WHO grades 3 or 4). For the study that used the daily questionnaire,(36) occurrence of CIN was assessed for five consecutive days from the start of CTX administration.

Of the nine studies that assessed the occurrence of CIN,(25, 27, 28, 30-34, 36) only three reported its occurrence rate.(28, 30, 34) The CIN occurrence rates were: 4.3%,(34) 21.8%,(28) and 23.3% (30) and the grand mean percentage rate was 9.9%.

Assessment of CIN severity – Six studies evaluated the severity of CIN.(21-24, 26, 35) In three studies,(21-23) CIN severity was assessed using a VAS (i.e., no nausea (0 mm) to the most

extensive nausea (100 mm)) before CTX administration, between 0 and 4 hours, and between 5 and 24 hours after CTX administration. In one study,(24) the severity of CIN was rated using a Likert scale (i.e., 0 = none, 1 = mild, 2 = moderate, 3 = severe). between 0 and 24 hours and between 2 and 5 days after CTX. While one study used NCICTC version 3 to assess CIN severity,(26) the timing of the assessment was not reported.(26) In one study,(35) the Memorial Symptom Assessment Scale (MSAS) was used to assess the severity of CIN once in seven days for each cycle of CTX administration.

Of the six studies that assessed the severity of CIN,(21-24, 26, 35) four reported its severity.(21-23, 35) Across three studies that used a VAS,(21-23) the weighted grand mean average CIN severity score was 12.7 for the observation period between the 5th hour and the 24th hour after CTX administration. In the study that used the MSAS,(35) the average CIN severity for 105 patients was 1.7.

Assessment of CIV occurrence – Fourteen studies evaluated the occurrence of CIV.(21-28, 30-34, 36) Three of these studies had patients report the number of vomiting and retching episodes in a daily diary immediately before CTX administration, between 0 and 4 hours, and between 5 and 24 hours after CTX administration.(21-23) In the six studies that used a diary to assess the occurrence of CIV,(24, 25, 27, 29, 31, 36) patients completed the diary for 24 hours(29) or for 5 days(24, 31, 36) following CTX administration. In two studies,(25, 27) patients documented any CIV event on an hourly basis for two days following CTX administration.

Of the five studies that used the NCICTC to assess CIV occurrence, four(26, 28, 32, 34) used version 3 and one(33) used version 4. In four of these studies,(26, 28, 32, 34) the occurrence of acute CIV was categorized as absent (i.e., grades 1 or 2) or present (i.e., grades 3 or 4). In two studies,(28, 32) based on patient documentation of any vomiting episode, delayed CIV was dichotomized as “yes” or “no”. In these two studies,(28, 32) the occurrence of CIV was

assessed daily for 5 days after CTX administration. In a third study,(26) CIV occurrence was assessed for 24 hours following CTX administration. In the fourth study that used NCICTC version 3,(34) CIV occurrence was assessed biweekly. In the study that used NCICTC version 4,(33) the occurrence of CIV was assessed at the oncology clinic prior to CTX administration and before each subsequent cycle. The occurrence of acute CIN was categorized as absent (i.e., grades 1 or 2) or present (i.e., grade 3).

Of the fourteen studies that evaluated the occurrence of CIV, ten reported its occurrence.(21-28, 30, 34) These occurrence rates ranged from 18.6%(26) to 40.0%(24) and the grand mean percentage was 14.2%.

Assessment of CIV severity – In the one study that used the MSAS to evaluate the severity of CIV,(35) it was assessed once in seven days for each cycle of CTX. CIV severity scores ranged from 0.0 ($\pm$ 0.0) to 0.3 ($\pm$ 0.7) with an average score of 0.25.

Analysis of genetic polymorphisms

Genotyping methods and statistical analyses – A variety of methods were used to identify genetic polymorphisms. Eight studies used restriction fragment length polymorphism (RFLP) and real time polymerase chain reaction (PCR) techniques to detect single nucleotide polymorphisms (SNPs).(21, 24, 25, 28, 29, 31, 35, 36) Other techniques used were: automated capillary DNA sequencing,(22, 23) multiplex PCR primer extension,(26) MegaBACE 1000 sequencer,(27) genotyping microarray,(30) and mass spectrometry.(32-34)

Across the sixteen studies, Chi square analysis was the predominant method used to evaluate for associations between a CINV phenotype and genotype.(22-28, 34) For multivariate analyses, logistic regression was used in six studies.(22, 30-32, 34, 36) Three studies used one-way analysis of variance (ANOVA) to evaluate for differences in CINV characteristics with respect to specific polymorphisms.(24, 29, 35) Two studies performed a Kaplan Meier log rank

test,(25, 27) two conducted a Cox proportional hazard regression analysis,(25, 33) and one performed the Cochran-Mantel-Haenzel test(31) to determine associations between genetic polymorphisms and antiemetic responses. Fourteen out of the sixteen studies evaluated Hardy Weinberg equilibrium.(22-27, 29-36)

Associations between CIN and genetic polymorphisms

Associations between occurrence of CIN and genetic polymorphisms – As shown in Table 1, nine studies evaluated for associations between the occurrence of CIN and a number of genetic polymorphisms.(25, 27, 28, 30-34, 36) The specific genes evaluated included: *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D*, and *HTR3E*;(25, 27) *ABCB1*;(28, 31-33, 36) ATP binding cassette subfamily C member 1 (*ABCC1*), ATPase copper transporting beta (*ATP7B*), and ATP binding cassette subfamily G member 2 (*ABCG2*);(33) *CYP2D6*;(28) dihydropyrimidine dehydrogenase (*DPYD*);(34) and general transcription factor IIE subunit 1 (*GTF2E1*)(33). In the two studies that evaluated for associations between the occurrence of CIN and polymorphisms in a number of serotonin receptor genes,(25, 27) no associations were found with any of the SNPs in *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D*, and *HTR3E*.

Five studies evaluated for associations between the occurrence of CIN and polymorphisms in *ABCB1*.(28, 31-33, 36) In the three studies that assessed rs1045642,(31, 32, 36) two found an association with the occurrence of CIN.(32, 36) Compared to patients who were homozygous or heterozygous for the common C allele, patients who were homozygous for the rare T allele had a decreased occurrence of CIN.

In two(32, 36) of the three studies that assessed for an association between the occurrence of CIN and *ABCB1* rs20325282,(31, 32, 36) compared to patients who were homozygous for the common G allele, patients who were heterozygous (GT/A) or homozygous for the rare allele (TT/A) had a decreased occurrence of CIN ($p = 0.012$ and $p = 0.021$, respectively). In the third

study,(31) patients who were homozygous for the rare T allele in this SNP were at increased risk for CIN ($p = 0.045$).

In the two studies that assessed for associations between the occurrence of CIN and *ABCB1* rs1128503,(33, 36) only one found that being homozygous for the rare C allele was associated with an increased occurrence of acute CIN ($p = 0.027$).(36) In one of the five studies that assessed *ABCB1*, a haplotype analysis was done.(28) Patients with the CTT haplotype for three SNPs in the *ABCB1* gene (i.e., rs1045642, rs20325282, rs1128503) experienced a decreased occurrence of acute CIN. However, this association did not reach significance ($p = 0.07$). In addition, compared with other *ABCB1* haplotypes, patients with the CTG haplotype experienced an increased occurrence of delayed CIN ($p = 0.02$).(28) In one study,(33) no associations were found between the occurrence of CIN and two SNPs in *ABCC1* (i.e, rs246240, rs2238476). However, patients with missense mutations in *ATP7B* rs1801244 (i.e., valine to leucine change) and *ABCG2* rs2231142 (i.e. glutamine to lysine change) were at an increased risk for CIN ($p = 0.027$ and $p = 0.045$ respectively).

In the one study that assessed for an association between the occurrence of CIN and polymorphisms in the drug metabolizing enzyme gene *CYP2D6* (i.e., rs16947, rs3892097, rs1065852),(28) no associations were found ($p = 0.12$). In another study that assessed for an association between the occurrence of CIN and a polymorphism in the *DPYD* enzyme gene,(34) patients with a splice donor variant in *DPYD*2A* rs3918290 (c.1905 + 1 G>A) were at an increased risk for CIN ($p = 0.007$). In a different study,(33) that assessed for an association between CIN and a polymorphism in the intronic region of the transcription factor *GTF2E1* gene, (rs447978, specific allele not reported), patients had a 78% decrease in odds of experiencing CIN (OR (dominant model) = 0.22, 95% CI = -2.52 to -0.49, $p = 0.004$). In a genome wide association study (GWAS) that evaluated a number of adverse events associated

with the administration of CTX,(30) no polymorphisms were found that were associated with the occurrence of CIN.

Associations between severity of CIN and genetic polymorphisms – As shown in Table 1, six studies evaluated for associations between the severity of CIN and polymorphisms in *HTR3A*,(23) *HTR3B*;(22) *HTR3C*;(26, 35) *ABCB1*;(24) catecholamine-o-methyltransferase enzyme (*COMT*);(35) *CYP2D6*;(21, 22) and guanidine triphosphate cyclohydrolase I (*GCH1*)(35). Of the four studies that evaluated for associations between the severity of CIN and polymorphisms in serotonin receptor genes,(22, 23, 26, 35) three(22, 23, 26) found no associations for any polymorphisms in *HTR3A*, *HTR3B*, and *HTR3C*. In one study,(35) being homozygous for the rare C allele for *HTR3C* rs6766410 was associated with decreased severity of acute CIN ($p = 0.04$). The association between the severity of CIN and *HTR3C* rs6807362 was not significant ($p = 0.08$).(35)

In the study that assessed for an association between CIN severity and *ABCB1* rs1045642,(24) being homozygous for the common C allele was associated with more severe acute CIN ($p = 0.044$). In contrast, no association was found between CIN severity and *COMT* rs4818 (p value not reported).(35) In one (22) of the two studies, that assessed for an association between the severity of CIN and the *CYP2D6* ultrarapid metabolizer (UM) allele, patients who were carriers of this allele had an increased risk for more severe CIN ($p = 0.03$). In the second study,(21) a similar trend was found but did not reach statistical significance. In the study that evaluated for associations between CIN severity and polymorphisms in *GCH1* (i.e., rs10483639, rs3783641, rs8007267),(35) the results were not significant (p values not reported).

Associations between CIV and genetic polymorphisms

Associations between occurrence of CIV and genetic polymorphisms – As shown in Table 1, fourteen studies(21-28, 30-34, 36) evaluated for associations between the occurrence of CIV and

a number of polymorphisms in *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D*, and *HTR3E*;(22, 23, 25-27) *ABCB1*;(24, 28, 31-33, 36) *ABCC1*, *ATP7B*, and *ABCG2*;(33) *CYP2D6*;(28) *DPYD*;(34) and *GTF2E1*(33).

In two studies,(23, 27) no associations were found between the occurrence of CIV and polymorphisms in *HTR3A* (i.e., rs1062613, rs1176722, rs1176719, rs2276303, rs909411, rs1176713). In one study,(22) being homozygous for -100_-102AAG deletion variant in *HTR3B* was associated with increased episodes of CIV ($p < 0.02$). In one(25) of the two studies that evaluated for associations between the occurrence of CIV and polymorphisms in *HTR3C*, patients who were homozygous for rare C allele in rs6766410 had a shorter time to first emetic event. In the second study,(26) none of the seven SNPs in *HTR3C* demonstrated a significant relationship with the occurrence of CIV. In another study,(27) no associations were found between the occurrence of CIV and polymorphisms in *HTR3D* (i.e., rs6443930, rs1000952) and *HTR3E* (i.e., rs5855015, rs7627615, rs56109847).

Six studies evaluated for associations between the occurrence of CIV and polymorphisms in drug transport pathway genes.(24, 28, 31-33, 36) While five studies assessed *ABCB1* rs1045642,(24, 28, 31, 32, 36) only three(24, 32, 36) found an association with the occurrence of CIV. Being homozygous for the rare T allele in rs1045642 was associated with a decreased occurrence of acute CIV ($p = 0.044$, $p = 0.002$, and $p = 0.016$, respectively). Of the three studies that evaluated for an association between the occurrence of CIV and *ABCB1* rs20325282,(31, 32, 36) in only one study,(31) was being homozygous for the rare T allele was associated with an increased likelihood of reporting the occurrence of CIV ($p = 0.045$). In contrast, in the other two studies,(32, 36) being homozygous for the rare T allele in rs20325282 was associated with a decreased likelihood of CIV ($p = 0.038$ and $p = 0.021$).

Two studies evaluated for an association between the occurrence of CIV and *ABCB1* rs1128503.(33, 36) While in one study, no association was found,(33) in the second study being homozygous or heterozygous for the rare C allele was associated with an increased number of episodes of vomiting ($p = 0.027$).(36) In another study,(28) patients who were carriers of the CTG haplotype in *ABCB1* (i.e., rs1045642, rs20325282, rs1128503) experienced an increased occurrence of delayed CIV ($p = 0.02$). In another study,(33) no associations were found between the occurrence of CIV and polymorphisms in a number of drug transport pathway genes (i.e., *ABCC1* rs246240 and rs2238476, *ABCG2* rs2231142, *ATP7B* rs1801244).

Two studies evaluated for associations between the occurrence of CIV and polymorphisms in drug metabolizing enzyme gene *CYP2D6*.(21, 28) While in one study, no association was found,(28) in the second study,(21) patients who were carriers of the UM allele for *CYP2D6* experienced an increased occurrence of acute CIV ($p < 0.03$).

One study investigated the association between the occurrence of CIV and a *DPYD* polymorphism. Patients with the splice donor variant *DPYD*2A* rs3918290 (c.1905 + 1 G>A) were at an increased risk for the occurrence of CIV ($p = 0.007$).(34) In the only study that evaluated for an association between the occurrence of CIV and a polymorphism in transcription factor gene *GTF2E1*,(33) no association was found with rs447978 (specific allele not reported). In a GWAS study,(30) no significant associations were found with the occurrence of CIV.

Association between severity of CIV and genetic polymorphisms – One study evaluated for associations between the severity of CIV and a number of genetic polymorphisms in *5-HTR3C*, *COMT*, and *GCH1* genes.(35) No associations were found between the severity of CIV and polymorphisms in *HTR3C* rs6766410 and rs6807362, *COMT* rs4818, and *GCH1* rs10483639, rs3783641, rs8007267.(35)

Associations between antiemetic efficacy and genetic polymorphisms

As shown in Table 2, twelve studies evaluated for associations between the efficacy of antiemetics and polymorphisms *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D*, and *HTR3E*;(22, 23, 25-28) *ABCB1*;(24, 28, 31, 32, 36) *CYP2D6*;(21, 22, 28) and *OCT1*(29).

In two studies,(23, 27) no associations were found between antiemetic efficacy and polymorphisms in *HTR3A*. In one study that included a haplotype analysis,(23) patients who were carriers of a CT haplotype in *HTR3A* (rs IDs not reported) were less likely to experience CIV and CIN with prophylactic antiemetic treatment ($p = 0.01$). In four studies,(22, 25, 27, 28) no associations were found between antiemetic efficacy and polymorphisms in *HTR3B* (rs1176744, rs45460698, rs4938058, rs7943062). In the two studies that assessed for an association between antiemetic efficacy and polymorphisms in *HTR3C*,(25, 26) only one (25) found that patients who were homozygous for the rare C allele in *HTR3C* rs6766410 had a shorter time to first emetic event within 24 hours of CTX administration ($p = 0.002$).

One study evaluated the association between antiemetic efficacy and polymorphisms in *HTR3D* and *HTR3E*.(27) Being homozygous for the rare C allele for *HTR3D* rs6443930 was associated with an increased likelihood of responding to serotonin antagonists ($p = 0.048$).(27) No associations were found between antiemetic efficacy and polymorphisms in *HTR3E* (rs5855015, rs7627615, rs56109847).

Six studies evaluated for associations between antiemetic efficacy and polymorphisms in drug transport pathway genes.(24, 28, 29, 31, 32, 36) Five studies evaluated for associations between antiemetic efficacy and polymorphisms in *ABCB1*.(24, 28, 31, 32, 36) In one study,(24) granisetron treated patients who were carriers of the rare T allele for *ABCB1* rs1045642 had a higher likelihood of a complete response in the acute phase. In another study of granisetron treated patients,(31) being homozygous or heterozygous for the rare T/A allele for *ABCB1*

rs20325282 was associated with a lower complete response rate in the acute phase. In another study of granisetron treated patients,(36) carriers of the TTT haplotype in *ABCB1* (i.e., rs1045642, rs20325282, rs1128503) had a higher complete response rate. In the same study, this finding was not observed in the ondansetron treated patients.(36) In two studies of patients treated with ondansetron,(28, 32) carriers of the CTG haplotype in *ABCB1* (i.e., rs1045642, rs20325282, rs1128503)(28) or carriers of the CG haplotype in *ABCB1* (i.e., rs1045642, rs20325282)(32) experienced an increased incidence of CIN and CIV.

One study evaluated for an association between antiemetic efficacy and polymorphisms in *OCT1*.(29) An *in vitro* assay demonstrated that polymorphisms in *OCT1* with amino acid substitutions (i.e., R61C, C88R, G401S, M420del, G465R) abolished tropisetron uptake. Plasma concentrations of tropisetron at 3 hours and 6 hours after administration and of ondansetron at 3 hours after administration were highest in patients who lacked a fully active *OCT1* allele ($p < 0.05$). Patients who lacked an active *OCT1* allele demonstrated a greater complete response ($p = 0.007$). This study controlled for the confounding effect of *CYP2D6* allele.

Three studies evaluated for associations between antiemetic efficacy and polymorphisms in the drug metabolizing enzyme gene *CYP2D6*.(21, 22, 28) While in one study,(28) no association was found in the other two studies,(21, 22) patients who were carriers of three active *CYP2D6* alleles (i.e., UMs) experienced decreased complete control of CIN and CIV after tropisetron and ondansetron administration. In one study,(21) patients with no active allele for *CYP2D6* (i.e., poor metabolizers (PMs)) had significantly higher serum concentrations of tropisetron and demonstrated greater complete control of CIN and CIV than patients with three active *CYP2D6* alleles ($p < 0.03$).

DISCUSSION

This comprehensive review summarizes findings from sixteen studies that evaluated for associations between the occurrence and/or the severity of CINV, as well as antiemetic efficacy, and polymorphisms in a variety of candidate genes. As shown in Tables 1 and 2, the majority of these genes were selected because they are involved in the mechanisms of CINV or in the major drug transport or drug metabolism pathways.

Serotonin pathway and CINV

Across the four CINV phenotypes (i.e., CIN occurrence and severity, CIV occurrence and severity), polymorphisms in five serotonin receptor genes were evaluated. This pathway was chosen because serotonin plays a major role in the development of CINV. Serotonin is released from enterochromaffin cells in the visceral mucosa following the administration of CTX. Serotonin activates 5-HT3 receptors on the vagus nerve which stimulates the medial nucleus of the solitary tract (NTS) and the dorsal vagal complex (DVC) in the medulla. This stimulation of the NTS and DVC signals vagal efferent fibers to produce retro-peristaltic contractions in the intestine and contractions in the stomach followed by relaxation of the gastric fundus and the lower esophageal sphincter. This action leads to expulsion of stomach contents.(40)

The 5-HT3 receptor is a ligand gated ion channel that is made up of five subunits (i.e., HTR3A, HTR3B, HTR3C, HTR3D, HTR3E).(41) The serotonin antagonists selectively block the excitation of presynaptic 5-HT3 receptors on the vagus nerve and act on the area postrema to block afferent signals from the vagus nerve that result in CINV.(40, 42)

As shown in Table 1, across six studies(22, 23, 25-27, 35) that evaluated 22 SNPs in the serotonin receptor pathway, only one found an association between CIN severity(35) and two found an association with CIV occurrence(22, 25). For CIN severity, patients who were homozygous for rare C allele, in rs6766410 reported less severe CIN. This nonsynonymous SNP

causes a change in the amino acid sequence from lysine to arginine which may alter the structure of the HTR3C receptor.(35) In another study,(25) this SNP was associated with an increase in the occurrence of CIV. The other SNP associated with the increased occurrence of CIV was *HTR3B* rs45460698.(22) In one *in vitro* study,(43) this deletion was associated with increased activity in the promoter region of *HTR3B*. However, these results need to be interpreted with caution because only 1.2% of the patients in the study had this polymorphism.

Drug transport pathway and CINV

Across the four CINV phenotypes, polymorphisms in four drug transport genes were evaluated. ABCB1 is a transmembrane glycoprotein that is present on the cell membrane of gastrointestinal (GI) tract enterocytes and on the endothelial cells of the cerebral cortex.(44) ABCB1 limits intracellular absorption of CTX in the GI tract and restricts the entry of CTX into the central nervous system (CNS). Polymorphisms in *ABCB1* may cause conformational changes in its protein structure and affect its function.(45) This alteration may affect the absorption of CTX across the blood brain barrier which affects the occurrence and/or severity CINV.

ABCC1 and ABCG2 are transmembrane proteins that are part of the blood brain barrier and cause the efflux of CTX drugs such as taxanes.(33) ATP7B is an ATPase expressed in the liver and kidney and to a lesser extent in the brain. Higher levels of *ATP7B* mRNA expression are correlated with higher rates of efflux and accumulation of CTX agents (i.e., carboplatin, cisplatin, oxaliplatin) in the bloodstream.(46) Polymorphisms in *ABCC1*, *ABCG2*, and *ATP7B* may change the rate of efflux of CTX drugs that enter the blood brain barrier and cause variations in occurrence and/or severity of CINV.

As shown in Table 1, across six studies,(24, 28, 31-33, 36) that evaluated seven SNPs and one haplotype in the drug transport pathway, five found associations with CIN occurrence,(28, 31-33, 36) one found an association with CIN severity,(24) and five found associations with CIV

occurrence(24, 28, 31, 32, 36). The most consistent finding across the CINV phenotypes were for the *ABCB1* gene. For ABCB1 rs1045642, patients who were homozygous for the rare T allele had a decrease in CIN(32, 36) and CIV(24, 32, 36) occurrence, as well as CIN severity,(24). While this synonymous SNP does not change the amino acid sequence, it significantly decreases ABCB1 function.(36)

The findings regarding *ABCB1* rs20325282 are inconsistent. In two studies,(32, 36) the occurrence of both CIN and CIV were decreased in patients who were homozygous for the rare T allele. In another study,(31) the exact opposite associations were found. *ABCB1* rs203252832 is a tri-allelic polymorphism where G is the common allele and A or T are the two possible rare variants. This nonsynonymous SNP causes a change in amino acid sequence from alanine to serine in the case of the rare A allele or threonine in the case of the rare T allele which may alter ABCB1 protein structure and/or function.(44)

Only one study found a positive association between *ABCB1* rs1128503 and occurrence of CIN and CIV.(36) While this synonymous SNP does not change the amino acid sequence of the protein, it may be in a linkage disequilibrium with another SNP that affects ABCB1 function. In one study,(28) patients with the CTG haplotype in *ABCB1* had an increase in the number of delayed CINV episodes. In a single study,(33) that evaluated two nonsynonymous SNPs in different genes (i.e., *ATP7B* rs1801244, *ABCG2* rs2231142), both SNPs were associated with an increase in CIN occurrence. While one SNP (*ATP7B* rs1801244) changes the amino acid sequence with no functional consequence,(33) the other SNP (*ABCG2* rs2231142) reduces ABCG2 efflux activity.(47)

Drug metabolism pathway and CINV

Across the four CINV phenotypes, only one drug metabolizing gene (i.e., *CYP2D6*) was evaluated. CYP2D6 belongs to a family of cytochrome P450 isoenzymes that bio-transforms

drugs through oxidation. CYP2D6 is a heme containing membrane protein that is expressed in the liver, kidneys, and GI tract.(48) Approximately 5% to 10% of Caucasians lack the active *CYP2D6* allele and as a result are PMs of drugs. Approximately 2% of Caucasians have more than 2 copies of active *CYP2D6* allele and are UMs.(21)

As shown in Table 1, across three studies,(21, 22, 28) that evaluated three SNPs and an UM polymorphism with more than two active copies of the gene as a result of duplication in *CYP2D6*, one found an association with CIN severity(22) and one with CIV occurrence(21). Patients who had the UM *CYP2D6* allele reported an increased severity of CIN and an increased occurrence of CIV. This finding suggests that these patients may have metabolized their antiemetics more rapidly.(21)

Antiemetic efficacy and genetic polymorphisms

As shown in Table 2, across twelve studies,(21-29, 31, 32, 36) associations between antiemetic efficacy and 24 SNPs and one haplotype in serotonin receptor genes, eight SNPs and one haplotype in two drug transport genes, and five alleles (i.e., including PM and UM) in drug metabolism pathways were evaluated. Three studies found associations between antiemetic efficacy and two SNPs and one haplotype in serotonin receptor genes.(23, 25, 27)

Most of the patients who had a CT haplotype in *HTR3A* and who were treated with tropisetron and ondansetron reported no CINV episodes. These two SNPs located in the intronic region of *HTR3A* have no known function.(23) In one study,(25) patients who were homozygous for the rare C allele in *HTR3C* rs6766410 and were treated with ondansetron and dexamethasone were non-responders. This nonsynonymous SNP changes the amino acid sequence from lysine to asparagine in the cysteine-loop of the HTR3C receptor and may impair ondansetron binding to the serotonin receptor.(25) In another study,(27) patients who were homozygous for the rare C allele in *HTR3D* rs6443930 and treated with ondansetron and dexamethasone demonstrate

increased antiemetic efficacy. This nonsynonymous SNP causes a change in the amino acid sequence from glycine to alanine near the N-terminus of the protein and may alter HTR3D protein structure.(27)

Five studies found an association between drug transport pathway genes and antiemetic efficacy.(24, 28, 29, 32, 36) Patients who were homozygous for the rare T allele in *ABCB1* rs1045642 and treated with granisetron reported a decrease in CINV.(24) In another study,(31) patients who were homozygous for the rare T allele in *ABCB1* rs20325282 and treated with granisetron reported increased CINV events. In one study,(36) patients who were homozygous for rare C allele in *ABCB1* rs1128503 and treated with granisetron reported increased CINV episodes. These SNPs may affect the level of ABCB1 gene expression or alter the structure of ABCB1 causing a change in granisetron binding to ABCB1.(36)

Patients with CG haplotype in *ABCB1* rs1045642 and rs20325282(32) or with TTT haplotype in *ABCB1* rs1045642, rs20325282, and rs1128503(36) and treated with granisetron demonstrated less complete control in the case of the CG haplotype and higher complete control for the TTT haplotype. Patients with the CTG(28) or the TTT(36) haplotypes in *ABCB1* and treated with ondansetron experienced less complete control. Given that the half-life of ondansetron is shorter than granisetron this difference may contribute to the findings for carriers of TTT haplotype.(49) The role of CG and CTG haplotypes in decreased complete control is not clear.(32, 36)

One study investigated the role of *OCT1* in the cellular uptake of tropisetron and ondansetron and its influence on the drug's therapeutic efficacy.(29) *OCT1* is one of the most abundantly expressed drug transport genes in the liver. It synthesizes OCT1, a plasma membrane protein that is critical for the elimination of many endogenous small organic cations, drugs, and toxins.(50) Polymorphisms in the exon region of *OCT1* were analyzed to determine if changes in

the amino acid sequence could impact drug transport function and influence cellular uptake of these antiemetics.(29) The *in vitro* and *in vivo* data suggest that concentrations of ondansetron were highest in patients who lacked the active *OCT1* allele and concentrations of ondansetron decreased with increases in number of active *OCT1* alleles. Patients who lacked active *OCT1* allele had higher plasma concentration of ondansetron and tropisetron. Patients who had active *OCT1* alleles vomited more frequently.

Drug-drug interactions may influence OCT1 function and contribute to inter-individual variability in hepatic uptake of tropisetron and ondansetron. CTX drugs like oxaliplatin but not carboplatin are substrates for OCT1.(29) Additional SNPs in *OCT1* discovered recently may influence the loss of function of OCT1.(50) Further investigation is required to understand the role of OCT1 in antiemetic efficacy.

In the two studies that found an association between drug metabolizing pathway genes and antiemetic efficacy, patients with three active CYP2D6 alleles referred to as the UM group who were treated tropisetron and ondansetron reported an increase in CINV episodes. In one study,(21) patients with no active *CYP2D6* alleles, (i.e., PMs) and treated with tropisetron and ondansetron, reported decreased number of CINV episodes. Since serum concentrations of tropisetron were highest in the PM group, it was considered a protective allele.(21)

Limitations of the sixteen studies

Sample size - Across the sixteen studies, the sample sizes ranged from 64 to 2886, with the majority of studies having a sample size of approximately 200 patients. None of the studies reported a power analysis based on the number of SNPs evaluated. Sample size selection for a candidate gene analysis depends on the number of SNPs analyzed, effect size of the SNPs, their allelic frequency, and the extent to which the SNPs are in linkage disequilibrium.(51) Of the 49 SNPs and one haplotype evaluated for associations with CINV, only 11 were statistically

significant. Of the 37 SNPs and two haplotypes evaluated for associations with antiemetic efficacy, only 10 were statistically significant. One reason for the lack of consistent findings across the sixteen studies is the relatively small sample sizes.

Allelic frequencies for *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D*, *HTR3E*, *ABCB1*, and *CYP2D6* genes differ among various ethnic populations. While these sixteen studies were conducted in nine different countries, most of them did not report patients' ethnicity and none reported if ancestry informative markers (AIMs) were used to control for these differences. Again, the failure to control for genomic estimates of race/ethnicity may contribute to the inconsistent findings. Most studies did not control for differences in phenotypic characteristics prior to the evaluation of associations between the various CINV phenotypes and genetic polymorphisms. In addition, most studies did not control for variations in the same gene.

Sample characteristics – Across the sixteen studies, patients varied in their cancer diagnoses. While in some studies, patients had a single cancer diagnosis, in other studies patients were heterogeneous in terms of their cancer diagnosis. Some studies recruited only female patients and one study recruited only male patients. Across the sixteen studies patients' ages ranged from 14 years to 86 years. The studies were rather diverse in the types of CTX as well as the antiemetic regimens that were evaluated. Diversity in sample characteristics across these studies may have contributed to inconsistent findings.

CINV assessment – While a variety of instruments can be used to assess CINV, no gold standard assessment tool is available. While some instruments, like the Morrow Assessment for Nausea and Vomiting (MANE) assess the frequency and severity of acute and anticipatory CINV,(52) others like the MASCC Antiemesis Tool (MAT) evaluate the occurrence and duration of acute and delayed CINV.(53)

While these two valid and reliable CINV tools are available, neither was used in any of the sixteen studies in this review. The majority of the studies used a VAS, the NCICTC and/or a patient diary to assess one or more of the CINV phenotypes. None of the studies reported on the validity and reliability of the VAS or the patient diary. The NCICTC does not evaluate the frequency of CIN. NCICTC version 3 assesses CIN for the first 24 hours and version 4 does not indicate the timing for the CIN assessment.

CONCLUSIONS

To date, between 13% to 60% of oncology patients experience CINV.(1-3) While sixteen studies have attempted to understand associations between various CINV phenotypes and polymorphisms in a number of candidate genes very few definitive conclusions can be drawn from these data due to the limitations enumerated above. As noted in Table 3, a number of areas warrant consideration in future research including adequately powered studies for the specific genomic analyses that are purposed; more rigorous phenotyping of CINV; evaluation of additional mechanisms that underlie CINV and antiemetic efficacy; and evaluation of changes in gene expression and epigenetics that contribute to the CINV phenotype and antiemetic efficacy.

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Figure 2.1 – PRISMA flow diagram to determine studies on associations between chemotherapy-induced nausea and vomiting phenotypes and candidate gene polymorphisms. Reprinted with permission from[20]

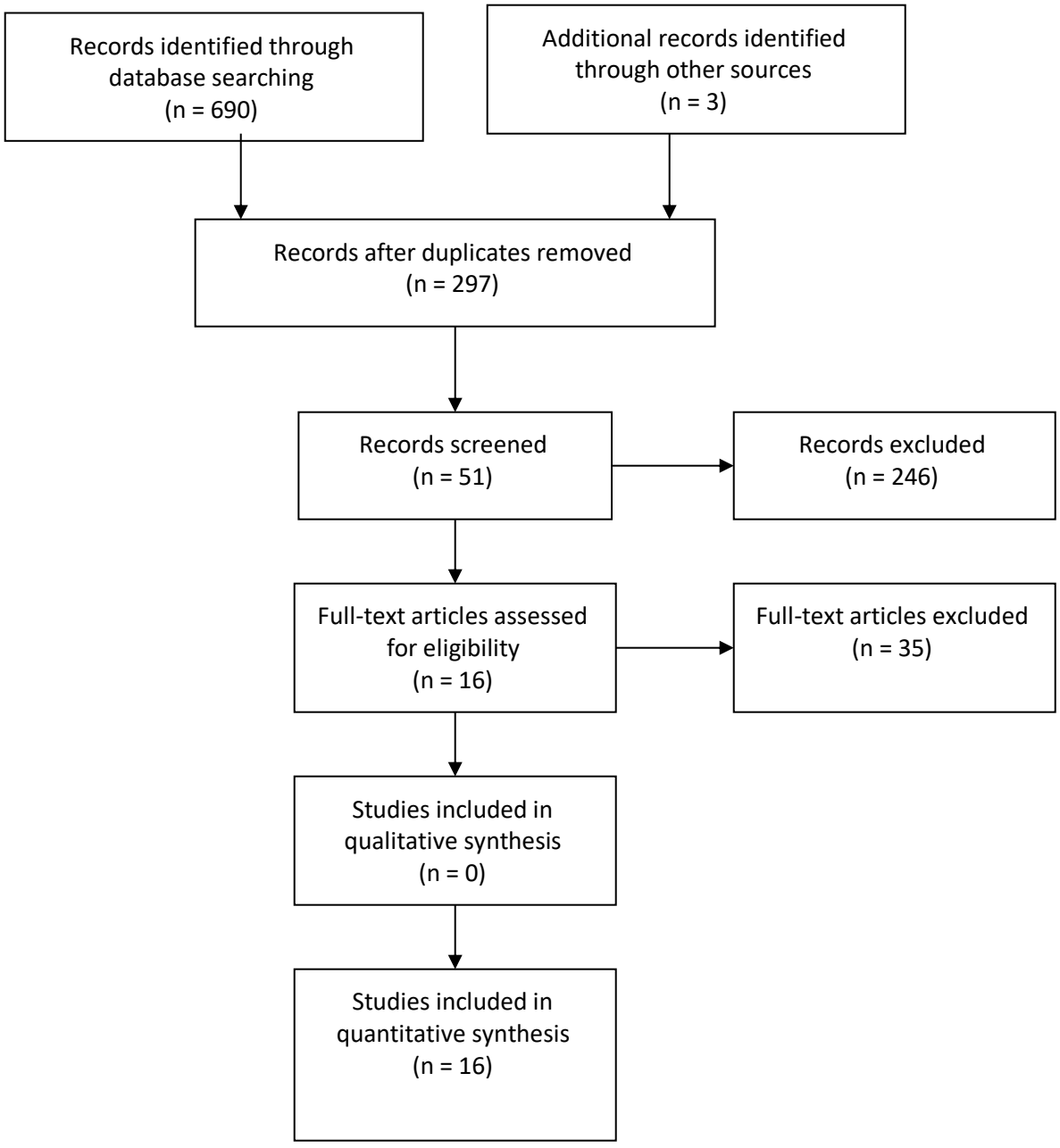

Table 2.1 – Summary of Findings on Associations Between Chemotherapy-Induced Nausea and Vomiting Phenotypes and Candidate Gene Polymorphisms

Gene	SNP	CIN Occurrence	CIN Severity	CIV Occurrence
		Findings	Findings	Findings
Serotonin receptor genes				
HTR3A	rs1062613	No association[27]	No association[23]	No association[23,27]
	rs1176722	No association[27]	No association[23]	No association[23,27]
	rs1176719		No association[23]	No association[23,27]
	rs2276303		No association[23]	No association[23,27]
	rs909411		No association[23]	No association[23,27]
	rs1176713		No association[23]	No association[23,27]
HTR3B	rs1176744	No association[25]	No association[22]	No association[25]
	rs45460698 (100_102AAG deletion)	No association[27]		↑ for homozygous variants[22]
HTR3C	rs6766410	No association[25]	↓ for rare allele[35]	↑ for rare allele[25]
	rs6807362	No association[25]	No association[35]	No association[25]
	1651 C>T		No association[26]	No association[26]
	3885 C>T		No association[26]	No association[26]
	3894 C>A		No association[26]	No association[26]
	6342 C>T		No association[26]	No association[26]
	7051 G>A		No association[26]	No association[26]
	7082 C>T		No association[26]	No association[26]
	7142 G>C		No association[26]	No association[26]
HTR3D	rs6443930	No association[27]		No association[27]
	rs1000952	No association[27]		No association[27]
HTR3E	rs5855015	No association[27]		No association[27]
	rs7627615	No association[27]		No association[27]
	rs56109847	No association[27]		No association[27]
Drug transport genes				
ABCB1	rs1045642	↓ for rare allele[32, 36] No association[31]	↓ for rare allele[24]	↓ for rare allele[24, 32, 36] No association[28, 31]
	rs20325282	↓ for rare allele[32, 36] ↑for rare allele[31]		↓ for rare allele[32, 36] ↑for rare allele[31]
	rs1128503	↑ for rare allele[36] No association[33]		↑for rare allele[36] No association[33]
	Haplotype rs1045642 + rs20325282 + rs1128503	↓ CTT haplotype NS[28] ↑ CTG haplotype[28]		↑ CTG haplotype[28]
ABCC1	rs246240	No association[33]		No association[33]
	rs2238476	No association[33]		No association[33]
ABCG2	rs2231142	↑ for Q to K change		No association[33]
ATP7B	rs1801244	↑ for V to L change		No association[33]
Drug metabolizing genes				
CYP2D6	rs16947	No association[28]		No association[28]
	rs3892097	No association[28]		No association[28]
	rs1065852	No association[28]		No association[28]
	(CYP2D6*1 + duplicate allele)		↑ for UM allele[22] ↑ for UM allele NS[21]	↑ for UM allele[21]

Gene	SNP	CIN Occurrence	CIN Severity	CIV Occurrence
		Findings	Findings	Findings
Enzyme genes				
COMT	rs4818		No association[35]	
DPYD	rs3918290	↑ for splice variant[34]		↑ for splice variant[34]
GCH1	s10483639		No association[35]	
	rs3783641		No association[35]	
	rs8007267		No association[35]	
Transcription factor gene				
GTF2E1	rs447978	↓ for intronic region SNP[33]		No association[33]
Genome Wide Association Study				
	rs10182133	No association[30]		
	rs2060645	No association[30]		
	rs6815391	No association[30]		
	rs7094179	No association[30]		
	rs9300811	No association[30]		
	rs2389972	No association[30]		
	rs10158985	No association[30]		
	rs851974	No association[30]		
	rs2739171	No association[30]		
	rs724975	No association[30]		

Blank box: Phenotype not studied

Abbreviations: ↑ = measured increased occurrence of CIN/CIV in comparison to reference allele, ↓ = measured decreased occurrence of CIN/CIV in comparison to reference allele, ABCB1 = ATP binding cassette subfamily B member 1, ABCC1 = ATP binding cassette subfamily C member 1, ABCG2 = ATP binding cassette subfamily G member 2, ATP7B = ATPase copper transporting beta, CIN = chemotherapy induced nausea, COMT = catecholamine-o-methyltransferase enzyme, CYP2D6 = cytochrome P450 family 2 subfamily D member 6, DPYD = dihydropyrimidine dehydrogenase, GCH1 = guanidine triphosphate cyclohydrolase I enzyme, GTF2E1 = general transcription factor IIE subunit 1, HTR3A = 5-hydroxytryptamine 3A receptor, HTR3B = 5-hydroxytryptamine 3B receptor, HTR3C = 5-hydroxytryptamine 3C receptor, HTR3D = 5-hydroxytryptamine 3D receptor, HTR3E = 5-hydroxytryptamine 3E receptor, K = Lysine, L = Leucine, NS = not significant, Q = Glutamine, UM = ultrarapid metabolizers, V = valine

Table 2.2 – Summary of Findings on Associations Between Antiemetic Treatment Efficacy and Candidate Gene Polymorphisms

Gene	SNP	Findings
Serotonin receptor genes		
HTR3A	rs1062613	No association[23, 27]
	rs1176722	No association[23, 27]
	rs1176719	No association[23, 27]
	rs2276303	No association[23, 27]
	rs909411	No association[23, 27]
	rs1176713	No association[23, 27]
	CT haplotype (8046 T > C and 10627 G >T)	↓ CINV occurrence in tropisetron and ondansetron treated patients [23]
HTR3B	rs45460698	No association[27, 28]
	rs1176744	No association[25]
	rs4938058	No association[25, 28]
	rs7943062	No association[25, 28]
HTR3C	rs6766410	↑ CIV episodes associated with rare allele in ondansetron and dexamethasone treated patients[25]
	rs6807362	No association[25]
	1651 C>T	No association[26]
	3885 C>T	No association[26]
	3894 C>A	No association[26]
	6342 C>T	No association[26]
	7051 G>A	No association[26]
	7082 C>T	No association[26]
	7142 G>C	No association[26]
HTR3D	rs6443930	↓ CINV occurrence for rare allele in ondansetron and dexamethasone treated patients [27]
	rs1000952	No association[27]
HTR3E	rs5855015	No association[27]
	rs7627615	No association[27]
	rs56109847	No association[27]
Drug transport genes		
ABCB1	rs1045642	↓ CINV occurrence in granisetron treated patients with rare allele[24]
	rs20325282	↑ CIV occurrence in granisetron treated patients homozygous (TT) or heterozygous (TA) for rare allele[31]
	rs1128503	↑ CIV occurrence in granisetron treated patients with rare allele[36]
	Haplotype rs1045642 + rs20325282 + rs1128503	↑ CINV occurrence in ondansetron treated patients with CG haplotype[32] ↑ CINV occurrence in ondansetron treated patients with CTG haplotype[28] ↓ CINV occurrence in granisetron treated patients with TTT haplotype[36]
OCT1	R61C	↓ CINV occurrence in tropisetron treated patients who lack active *OCT1* allele[29]
	C88R	
	G401S	
	M420del	
	G465R	
Drug metabolizing gene		
CYP2D6	rs16947	No association[28]
	rs3892097	No association[28]

Gene	SNP	Findings
CYP2D6	rs1065852	No association[28]
	UM (CYP2D6*1 + duplicate allele)	↑ CINV occurrence in tropisetron and ondansetron treated patients with three active alleles [21, 22]
	PM (Two alleles of CYP2D6*3 CYP2D6*4 CYP2D6*5 CYP2D6*6)	↓ CINV occurrence and ↑ serum tropisetron concentration in patients with no active alleles [21]

Abbreviations: ↑ = measured increased antiemetic efficacy, ↓ = measured decreased antiemetic efficacy, A = adenine, ABCB1 = ATP binding cassette subfamily B member 1, C88R = cysteine88-to-arginine, C = Cytosine, CINV = chemotherapy-induced nausea and vomiting, CIV = chemotherapy-induced vomiting, CYP2D6 = cytochrome P450 family 2 subfamily D member 6, G = guanine, G401S = glycine401-to-serine, G465R = glycine465-to-arginine, HTR3A = 5-hydroxytryptamine 3A receptor, HTR3B = 5-hydroxytryptamine 3B receptor, HTR3C = 5-hydroxytryptamine 3C receptor, HTR3D = 5-hydroxytryptamine 3D receptor, HTR3E = 5-hydroxytryptamine 3E receptor, M420del = deletion of methionine420, OCT1 = organic cation transporter protein, PM = poor metabolizers, R61C = arginine61-to-cysteine, T = thymine, UM = ultrarapid metabolizer

Table 2.3 - Directions for Future Research

Sample selection

- Control for genomic estimates of race/ethnicity
- Include sample size that provides adequate power for evaluating selected SNPs

CINV assessment

- Use valid and reliable instruments to characterize the CINV phenotypes (e.g., MANE)
- Determine the optimal timing for CINV measures to capture anticipatory, acute, and delayed CINV phenotypes.

Mechanistic considerations for candidate gene selection

- Evaluate additional pathways involved in the development of CINV (e.g., NK-1 receptor, dopamine receptor activation pathways).
- Evaluate additional pathways involved in antiemetic efficacy (e.g., drug metabolizing enzyme pathways other than CYP2D6)

Other types of genomic analyses

- Evaluate for changes in gene expression that contribute to anticipatory, acute and delayed CINV
- Evaluate for epigenetic changes that contribute to anticipatory, acute and delayed CINV

Abbreviations: CINV = chemotherapy-induced nausea and vomiting, CYP2D6 = cytochrome P450 family 2 subfamily D member 6, MANE = morrow assessment for nausea and vomiting, NK-1 = neurokinin-1, SNPs = single nucleotide polymorphisms

Supplementary Table 2.1 – Summary of studies on candidate gene polymorphisms to explain inter-individual differences in chemotherapy-induced nausea and vomiting

Author, Year Purpose, Study Design, Emetogeniciy of CTX, Major outcome(s)	Gene(s) Classified by Function[+]	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Kaiser et al. 2002 Purpose: Investigate whether the efficacy of antiemetic treatment with ondansetron and tropisetron depends on *CYP2D6* genotype Design: Prospective, cohort study CTX: Moderate to high emetogenicity Cyclophosphamide – 98 patients Cisplatin – 27 patients Carboplatin – 29 patients Miscellaneous CTX – 116 patients Glucocorticoids – 151 patients Major outcome(s): Relationship between number of episodes of vomiting and *CYP2D6* genotypes	Drug metabolizing enzyme *CYP2D6* Specific SNPs not reported	N = 270 Age: 53.7 ± 13.3 years Gender: 43.0% male Diagnosis: Breast cancer = 32.5% Lung cancer = 15.4% Non-Hodgkin's lymphoma = 14.2% Multiple myeloma = 4.9% Hodgkin's disease = 4.9% Other = 28.1% Setting: Outpatient and inpatient Antiemetic treatment: Standardized regimen of tropisetron and ondansetron	Assessment of CINV: Nausea: VAS 0-100 mm scale Vomiting: Daily diary of number of vomiting and retching episodes Timing of CINV assessment: Before CTX administration, between 0-4 hours and 5–24 hours after CTX administration Genotyping methods: PCR-RFLP and ABI 373A automated sequencer Statistical analyses: Mann-Whitney U test to determine association between *CYP2D6* genotype and mean severity of nausea as well as mean number of emetic episodes for 0-4 hours after CTX and 5-24 hours after CTX	Of 270 patients, 22.1% experienced CIV and 35.9% experienced CIN Patients on glucocorticoids were less likely to experience nausea (73.6% vs 51.8%, $p < 0.001$) Patients on highly emetogenic CTX without glucocorticoids experienced a two-fold higher intensity of nausea and vomiting in the 4 hours after CTX administration (mean, 12.8% vs 6.8%, $p < 0.02$) *CYP2D6* genotyping revealed that: 7.8% of patients were deficient for the *CYP2D6* gene (PM), 32.6% had one active allele, 58.1% had two active alleles (EM), and 1.5% had three active alleles (UM)	Strengths: Relatively large sample Conservative inclusion and exclusion criteria Nausea and vomiting assessed simultaneously Limitations: Confounding variables such as gender, age, alcohol intake, anxiety, and depression were not accounted for in the analysis Hardy Weinberg equilibrium for *CYP2D6* genotype frequency not reported

Major outcome(s):	Statistical analyses:	Major Findings:
Relationship between severity of nausea and *CYP2D6* genotypes Relationship between blood concentrations of tropisetron and *CYP2D6* genotypes	Mann-Whitney U test to determine association between *CYP2D6* genotype and tropisetron serum concentration 3 to 6 hours after administration Kruskal-Wallis test to determine differences in mean number of episodes of nausea and vomiting between patients who did and did not receive glucocorticoids	UMs for *CYP2D6* had higher mean number of vomiting episodes 4 hours after CTX (2.3 ± 2.5 vs 0.2 ± 1.0, $p < 0.001$) and at 5-24 hours after CTX (3.3 ± 3.5 vs 0.8 ± 2.4, $p < 0.03$) compared to other three groups. Mean number of episodes of severe nausea in UMs was higher but not statistically significant at 4 hours and between 5- 24 hours after CTX compared to the other three groups PMs had the highest serum concentrations of tropisetron compared to the other three groups ($p < 0.03$)

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Trembley et al. 2003 Purpose: Analyze variations in *5-HT3B receptor* genes to explain differences in patients' responses to antiemetic treatment Design: Prospective, cohort study CTX: Moderate to high emetogenicity Cyclophosphamide – 91 patients Cisplatin – 25 patients Carboplatin – 27 patients Miscellaneous CTX – 99 patients Glucocorticoids – 141 patients Major outcome(s): Relationship between number of episodes of vomiting and genetic variations Relationship between severity of nausea and	Serotonin receptor *5-HT3B receptor* Specific SNPs not reported Drug metabolizing enzyme *CYP2D6* Specific SNPs not re ported	N = 242 Age: 53.3 ± 13.6 years Gender: 43.0% male Diagnosis: Breast cancer = 32.0% Lung cancer = 16.0% Non-Hodgkin's lymphoma = 15.1% Hodgkin's disease = 5.5% Multiple myeloma = 4.6% Ovarian cancer = 4.1% Other = 22.7% Setting: Outpatient and inpatient Antiemetic treatment: Standardized regimen of tropisetron and ondansetron	Assessment of CINV: Nausea: VAS 0-100 mm scale Vomiting: Daily diary of number of vomiting and retching episodes Timing of CINV assessment: Before CTX administration, between 0-4 hours and 5–24 hours after CTX administration Genotyping methods: Automated capillary DNA sequencing of *5HT3 receptor* and *CYP2D6* genes Statistical analyses: Differences in genotype frequencies by Chi Square or FE tests Logistic regression with vomiting as dependent variable and age, gender, genotypes for *5HT-3B* and *CYP2D6*, and treatment with glucocorticoids	Of the 233 patients, 22.7% reported CIV and 35.9% reported CIN within the first 24 hours after CTX The mean number of vomiting episodes for patients who experienced CIV was 2.9 (range, 1 to 10) in the first observation period and 4.0 (range, 1 to 22) in the second observation period Mean percentage rates for CIN in first observation period was 39.2% (range, 21% - 74%) and in the second observation period was 46.3% (range, 21% to 98%) Homozygotes for the *-100*_-102AAG deletion variant in *5-HT3B receptor* gene had significantly more episodes of acute vomiting	Strengths: Relatively large sample Conservative inclusion and exclusion criteria Emetogenic level of CTX was similar for all patients Nausea and vomiting assessed simultaneously Limitations: Confounding variables such as gender, age, alcohol intake, anxiety, and depression were not accounted for in the analysis Low frequency of patients who are UMs (~2%) and homozygous for -100_-102AAG deletion polymorphism (1.3%)

Major outcome(s):	Statistical analyses:	Major Findings:
genetic variations Relationship between genotype and the pharmacokinetics of antiemetics	as independent variables	UMs for *CYP2D6* had higher turnover of ondansetron and tropisetron and had more severe acute nausea and vomiting

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Kaiser et al. 2004 Purpose: Investigate the relationship between polymorphisms in the *5HT3A receptor* gene and the intensity of nausea and vomiting Design: Prospective, cohort study CTX: Moderate to high emetogenicity Cyclophosphamide – 91 patients Cisplatin – 25 patients Carboplatin – 27 patients Miscellaneous CTX – 99 patients Glucocorticoids – 141 patients Major outcome(s): Relationship between number of episodes of vomiting and *5-HT3A receptor* polymorphisms Relationship between severity of nausea and *5-HT3A receptor* polymorphisms	Serotonin receptor *5-HT3A receptor* rs1062613 rs1176722 rs1176719 rs2276303 rs909411 rs1176713 While additional SNPs were analyzed rs IDs were not reported	N = 242, data analyzed for 233 patients Age: 53.3 ± 13.6 years Gender: 43.0% male Diagnosis: Breast cancer = 32.0% Lung cancer = 16.0% Non-Hodgkin's lymphoma = 15.1% Hodgkin's disease = 5.5% Multiple myeloma = 4.6% Ovarian cancer = 4.1% Other = 22.7% Setting: Outpatient and inpatient Antiemetic treatment: Standardized regimen of tropisetron and ondansetron	Assessment of CINV: Nausea: VAS 0-100 mm scale Vomiting: Daily diary of number of vomiting and retching episodes Timing of CINV assessment: Before CTX administration, between 0-4 hours and 5–24 hours after CTX administration Genotyping methods: Capillary DNA sequencer Statistical analyses: Chi square test used to evaluate for differences in the frequency distribution of genotypes and haplotypes between patients who did and did not experience CINV Kruskal-Wallis test used to evaluate for differences in the number of episodes of vomiting and severity of nausea	Of 233 patients, 23.7% experienced CIV and 35.9% experienced CIN No significant association between *5-HT3A receptor* gene polymorphisms and mean number of emetic episodes No significant association between *5-HT3A receptor* gene polymorphisms and mean severity of nausea Percentage of patients experiencing nausea and/or vomiting with prophylactic antiemetic treatment was independent of the emetogenic level of CTX Patients with haplotype 2 of the *HT3A receptor* gene were more likely not to experience vomiting compared to patients without	Strengths: Conservative inclusion and exclusion criteria Nausea and vomiting assessed simultaneously Limitations: Confounding variables such as anxiety and depression were not accounted for in the analysis Larger sample size needed to determine association between haplotype frequency and acute CINV

Statistical analyses:	Major Findings:
between/among the genotype groups for each SNP	this haplotype (93% vs 7%, p = 0.01)

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Babaoglu et al. 2005 Purpose: Investigate association between *ABCB1* 3435C>T genotype and antiemetic efficacy of 5-HT3 receptor antagonists in cancer patients receiving CTX Design: Prospective, cohort study CTX: Moderate to high emetogenicity Cisplatin or carboplatin –37 patients Cyclophosphamide – 142 patients Anthracyclines – 161 patients Glucocorticoids – 189 patients Major outcome(s): Relationship between antiemetic efficacy and polymorphisms in *ABCB1* rs1045642 Relationship between polymorphisms	Transporter protein *ABCB1* rs1045642 (3435 C>T)	N = 216 Age: 46.1 ± 10.7 years Gender: 25.0% male Diagnosis: Breast cancer = 63.0% Lymphoma = 14.8% Lung cancer = 10.2% Other = 12.0% Setting: Outpatient Antiemetic treatment: Standardized regimen of tropisetron, ondansetron, or granisetron	Assessment of CINV: Nausea: Self-report chart for timing and severity Vomiting: Self-report chart for number of vomiting episodes Timing of CINV assessment: Between 0-24 hours (acute phase) and 2–5 days (delayed phase) after CTX administration Genotyping methods: PCR-RFLP and TaqMan based real time PCR Statistical analyses: Chi square tests to evaluate for differences in demographic characteristics, allele frequencies, and efficacy of antiemetic treatments One-way ANOVA to evaluate for differences in demographic characteristics	In the total sample, 60% of the patients achieved complete control of CINV in the acute phase and 50% in the delayed phase regardless of antiemetic drug In the acute phase, the type of 5-HT3 receptor antagonists influenced the effect of genotype on antiemetic responses (i.e., patients who received granisetron had the most prominent responses) In the acute phase, for the entire sample, the complete control rate for nausea and vomiting was significantly higher in those homozygous for *ABCB1* 3435 T allele as compared to those carrying the C allele (p = 0.044)	Strengths: Relatively large sample Conservative inclusion and exclusion criteria Nausea and vomiting assessed simultaneously Demographic factors known to contribute to CINV such as age, gender, alcohol consumption and motion sickness were evaluated Distribution of CTX and 5-HT3 antagonist regimens was similar across the three *ABCB1* 3435 C>T genotype groups Limitations: Confounding variables such as anxiety and depression were not accounted for in the analysis

Major outcome(s):	Statistical analyses:	Major Findings:
in *ABCB1* rs1045642 and complete control rates for acute and delayed nausea and vomiting	among the three genotype groups	In the granisetron treated patients, the complete response rates in the acute phase were 99% in TT patients in comparison with TC patients (56.1%, p = 0.02) and CC patients (47.6%, p = 0.009) for *ABCB1* 3435 C>T genotype In patients treated with tropisetron or ondansetron, differences in complete response rates in the acute phase among the genotype groups did not reach statistical significance In the delayed phase, across the entire sample, the proportion of patients who had complete control of nausea and vomiting did not differ across genotype groups (p = 0.53)

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function[+]	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Fasching et al. 2008 Purpose: Correlate the occurrence of CINV with common SNPs in *5-HT3 receptor* genes Design: Prospective study CTX: Moderate emetogenicity 5-fluorouracil + epirubicin + cyclophosphamide – 33 patients Epirubicin + cyclophosphamide + either paclitaxel or docetaxel – 60 patients Epirubicin + paclitaxel + cyclophosphamide + methotrexate + fluorouracil –17 patients Major outcome(s): Association between complete emetic response in first 1 to 2 days of first CTX	Serotonin receptor *5-HT3B receptor* rs1176744 *5-HT3C receptor* rs6766410 rs6807362	N = 110 Age: 52.3 ± 10.4 years Gender: 100% female Diagnosis: Breast cancer = 100% Setting: Outpatient Antiemetic treatment: Standardized regimen of ondansetron and dexamethasone	Assessment of CINV: Nausea: diary Vomiting: diary Timing of CINV assessment: Hourly documentation of any event involving nausea or vomiting on days 1 and 2 of first CTX cycle Genotyping methods: Real time PCR for single SNPs in *5-HT3B receptor* and *5-HT3C receptor* genes Statistical analyses: Chi square test to determine association between complete response and genotype Kaplan-Meier curves for log-rank test to estimate time to antiemetic treatment failure for first cycle of CTX Cox proportional hazard regression	Of the 110 patients, 35 experienced CIV in the first 24 hours after receiving CTX No associations were found between complete emetic response and polymorphisms in *5-HT3B receptor* rs1176744 and *5-HT3C* receptor rs6807362 A higher percentage of patients who were homozygous for the rare allele (CC) in *5-HT3C receptor* rs6766410 were non-responders Kaplan-Meier estimates for time to first emetic event was significant for *5-HT3C receptor* rs6766410 with homozygotes for the rare C allele having the worst profile Cox proportional hazard regression	Strengths: Specific inclusion criteria to ensure a relatively homogenous sample of patients Attempts to gain insights into changes in protein function of 5-HT3B and 5-HT3C receptor(s) Limitations: Small sample size Unclear definition of nausea and vomiting One in three patients refused participation which suggests a selection bias

Major outcome(s):	Statistical analyses:	Major Findings:
infusion and genotype Association between time to first emetic episode and genotype Association between time to emetic treatment failure and genotype	analysis for time to antiemetic treatment failure in relation to different genotypes	analysis revealed that compared to patients who were homozygous or heterozygous for the common allele (AA or AC) in *5-HT3C receptor* rs6766410, patients who were homozygous for the rare allele in this SNP (CC) had a hazard ratio of 2.88 (95% CI, 1.46 – 5.67, p = 0.002) for the first emetic episode within 24 hours of CTX administration

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function[+]	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Hammer et al. 2010 Purpose: Correlate the occurrence of CINV with common SNPs in *5-HT3 receptor* genes Design: Prospective, cohort study CTX: Moderate emetogenicity 5-fluorouracil + epirubicin + cyclophosphamide – 33 patients Epirubicin + Cyclophosphamide – 60 patients Epirubicin -17 patients Major outcome(s): Association between complete emetic response in first 1 to 2 days of first CTX infusion and genotype Association between time to first emetic episode and genotype	Serotonin receptor *5-HT3A receptor* rs1062613 rs1176722 *5-HT3B receptor* rs45460698 *5-HT3D receptor* rs6443930 rs1000952 *5-HT3E receptor* rs5855015 rs7627615 rs56109847	N = 110 Age: <50 years – 45 patients 50-59 years – 37 patients >59 years – 28 patients Gender: 100% female Diagnosis: Breast cancer = 100% Setting: Outpatient Antiemetic treatment: Standardized regimen of ondansetron and dexamethasone	Assessment of CINV: Nausea: diary Vomiting: diary Timing of CINV assessment: Hourly documentation of any event involving nausea or vomiting on days 1 and 2 of first CTX cycle Genotyping methods: MegaBACE 1000 sequencer Statistical analyses: Hardy Weinberg equilibrium and genotype frequency determined by SNPassoc software package for R Chi square test to determine association between complete response and genotype Kaplan-Meier curves and log-rank test to estimate time to antiemetic	35 patients were non-responders and experienced acute CIV Patients younger than 50 years were more likely to experience vomiting ($p = 0.033$) No association between emetic episode and BMI ($p = 0.242$), smoking history ($p = 0.458$), alcohol intake ($p = 0.619$), or emetogenicity of CTX ($p = 0.082$) After controlling for multiple testing, no genetic associations were significant	Strengths: Specific inclusion criteria to ensure a relatively homogenous sample of patients Investigated a large number of polymorphisms in *5-HT3 receptor* subtypes to determine associations with CINV Genotype frequency and haplotype analysis were reported Limitations: Small sample size Unclear definition of nausea and vomiting Other confounding variables such as anxiety and depression were not accounted for in the analysis

Major outcome(s):	Statistical analyses:
Association between time to emetic treatment failure and genotype	treatment failure for first cycle of CTX

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function[+]	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Perwitasari et al. 2011 Purpose: Correlate the occurrence of CINV with common SNPs in *5-HT3B receptor*, *ABCB1*, and *CYP2D6* genes in patients treated with highly emetogenic CTX Design: Prospective, cohort study CTX: High emetogenicity Cisplatin (50 – 70 mg/m^2) – 183 patients Cisplatin (75 – 100 mg/m^2) – 19 patients Major outcome(s): Association between emetic response in first 1 to 5 days of first CTX infusion and *5-HT3B receptor*, *ABCB1*, and *CYP2D6* genotype Association between antiemetic drug	Serotonin receptor *5-HT3B receptor* rs45460698 rs4938058 rs7943062 Transporter protein *ABCB1* rs1045642 rs2032582 rs1128503 Drug metabolizing enzyme *CYP2D6* rs16947 rs3892097 rs1065852	N = 202 Age: 48.6 ± 9.6 years Gender: 93.1% female Diagnosis: Cervical cancer = 59.9% Ovarian cancer = 28.7% Nasopharyngeal cancer = 6.4% Vulva cancer = 3.4% Lung cancer = 1.6% Setting: Inpatient and outpatient Antiemetic treatment: Standardized regimen of ondansetron and dexamethasone for acute CINV Standardized regimen of metoclopramide administered for 5 days after CTX administration for delayed CINV	Assessment of CINV: Nausea: 0-100 mm VAS Acute nausea was the primary outcome and delayed nausea was the secondary outcome. They were categorized based on NCICTC v.3. Acute CIN was grouped as grade 1-2 or 3-4. Delayed CIN was categorized as dichotomous variable (yes/no) Vomiting: Daily record for number of vomiting episodes. Acute vomiting was the primary outcome and delayed vomiting was the secondary outcome. They were categorized based on NCICTC v.3. Acute CIV was grouped as grade 1-2 or 3-4. Delayed CIV was categorized as dichotomous variable (yes/no)	Of the 202 patients, 21.8% experienced acute nausea, 30.2% experienced acute vomiting and 38.6% patients experienced delayed nausea and/or vomiting Compared with the other haplotypes, patients with the CTG haplotype in *ABCB1*gene expressed more frequent grade 3 to 4 CINV (p = 0.02) The percentage of EMs and IMs for CYP2D6 phenotype was 59.9% and 32.7%, respectively in the sample No associations were found between phenotypes for *CYP2D6* and acute or delayed CINV	Strengths: Specific inclusion criteria to ensure a relatively homogenous sample of patients Conservative exclusion criteria Nausea and vomiting assessed simultaneously Limitations: Larger sample size needed to determine association between haplotype frequency of *5-HT3B receptor*, *ABCB1*, and *CYP2D6* genes and CINV Other confounding variables such as anxiety and depression were not accounted for in the analysis Hardy Weinberg equilibrium for genotype frequency not reported

Major outcome(s):

efficacy and *5-HT3B receptor*, *ABCB1*, and *CYP2D6* genotype

Timing of CINV assessment:

Daily documentation of any event involving nausea or vomiting from days 1 to 5 after CTX administration

Genotyping methods:
TaqMan based real time PCR

Statistical analyses:
Patients were categorized as PMs (i.e., poor metabolizers), IMs (i.e., intermediate metabolizers) or EMs (i.e., extensive metabolizers) based on whether the *CYP2D6* allele was defective, had decreased activity, or was active

Chi square test to evaluate association between patient characteristics and acute and delayed CINV

Chi square test to evaluate association between acute and delayed CINV and *5-HT3B receptor*, *ABCB1*, and *CYP2D6* genotypes

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Tzvetkov et al. 2012 Purpose: Determine whether *OCT1* mediated the cellular uptake of tropisetron and ondansetron and whether, and to what extent, genetic polymorphisms in *OCT1* contributed to the variability in pharmacokinetics and therapeutic efficacy of tropisetron and ondansetron in oncology patients Design: Prospective, cohort study CTX: Moderate to high emetogenicity Specific CTX regimens not reported Major outcome(s): Association between OCT1 overexpression and cellular uptake of	Organic cation transporter protein *OCT1* (i.e., *SLC22A1*) Evaluated common genetic polymorphisms associated with amino acid substitutions: R61C C88R G401S M420del G465R Drug metabolizing enzyme *CYP2D6* Specific SNPs not reported	N = 270 Age: 53.7 ± 13.3 years Gender: Not reported Diagnosis: Breast cancer = 32.0% Lung cancer = 15.4% Non-Hodgkin's lymphoma = 14.2% Hodgkin's disease = 4.9% Multiple myeloma = 4.9% Other = 28.1% Setting: Outpatient Antiemetic treatment: Standardized regimen of tropisetron and ondansetron Second sample of 60 patients received only ondansetron Age: 53.4 ± 13 years Diagnosis: Non-Hodgkin's lymphoma = 66%	Assessment of CINV: Nausea: Not assessed Vomiting: Patient diary for episode of acute emesis Timing of CINV Assessment: First 24 hours after CTX Genotyping methods: Single base primer extension, PCR-RFLP and TaqMan based real time PCR Statistical analyses: Student t test to evaluate for differences in intracellular concentrations of tropisetron in OCT1 – overexpressing cells compared to cells transfected with control plasmid (*in vitro*) One-way ANOVA to evaluate for differences in intracellular concentrations	Compared to cells transfected with control plasmid, the OCT1– overexpressing cells showed a 2.3-fold increase in intracellular accumulation of tropisetron OCT1 overexpression did not result in additional increase of intracellular ondansetron uptake Common *OCT1* polymorphisms found in Caucasians that cause amino acid substitutions (R61C, C88R, G401S, M420del or G465R) when expressed in HEK293 cells abolished tropisetron uptake. This *in vitro* experiment did not show any change in ondasetron uptake The plasma concentrations of tropisetron at	Strengths: Relatively large sample Design and execution of *in vitro* experiments to determine the correlations between tropisetron and ondansetron concentrations with *OCT1* genotypes and to determine if the direction of correlation was similar to *in vivo* plasma concentrations of tropisetron and ondansetron with the same *OCT1* genotypes Determination of the effect size of *OCT1* genotype on acute CIV episodes following the administration of tropisetron and ondansetron A second study sample to corroborate findings on the correlation between *OCT1* polymorphisms

Major outcome(s):	Diagnosis:	Statistical analyses:	Major Findings:	Strengths:
tropisetron and ondansetron *in vitro* Relationship between plasma concentration of tropisetron and ondansetron with *OCT1* genotype *in vivo* Relationship between mean episodes of vomiting, in patients on ondansetron and tropisetron, with *OCT1* genotype in the first 24 hours after CTX	Hodgkin's disease = 11% Multiple myeloma = 3.1% Lung cancer = 3.1% Other cancers = 16.8% Setting: Outpatient Antiemetic treatment: Standardized regimen of ondansetron	of tropisetron among overexpressed *OCT1* variants carrying the five common amino acid substitutions compared to wild-type *OCT1* (*in vitro*) One-way ANOVAs to evaluate for differences in plasma concentrations of tropisetron and ondansetron and number of vomiting episodes in relationship to the number of fully active *OCT1* alleles Linear regression analysis to evaluate the effects of *OCT1* genotypes on: plasma concentrations of tropisetron and ondansetron and antiemetic efficacy after controlling for the effects of *CYP2D6* polymorphisms	3 and 6 hours after administration and of ondansetron at 3 hours after administration were highest in the subgroups of patients lacking any fully active *OCT1* alleles and decreased with the increasing number of fully active *OCT1* alleles Patients lacking any fully active *OCT1* allele vomited more than three times less frequently than patients with one or two fully active *OCT1* alleles A mean of 0.8 episodes of vomiting was observed in patients with fully active *OCT1* compared to a mean of 0.08 episodes of vomiting in patients with one or two deficient *OCT1* alleles (p = 0.009) Of the 253 patients who received ondansetron, a mean of 0.37 episodes of vomiting was observed in the group lacking	and episodes of vomiting Limitations: Chemotherapy induced nausea was not assessed No attempt to determine association between chemotherapy induced nausea and *OCT1* and *CYP2D6* genotypes CTX regimens administered to patients were not reported Stage of cancer was not reported The study sample were restricted to Caucasians

Major Findings:

fully active *OCT1* compared with a mean of 1.27 episodes of vomiting observed in carriers with one or two fully active *OCT1* alleles ($p = 0.018$)

After adjusting for the effects of *CYP2D6* genotypes, plasma concentrations of tropisetron at 3 hours and 6 hours after administration ($p = 0.02$ and $p = 0.04$, respectively) depended on *OCT1* genotype

OCT1 genotype explained 8.1% of variance in tropisetron levels at 3 hours and 11.3% at 6 hours after administration

CYP2D6 genotype explained 9.4% of variance in tropisetron plasma levels at 3 hours and 12% of variance at 6 hours after administration

After adjusting for the effects of *CYP2D6* genotypes, plasma concentrations

Major Findings:

of ondansetron depended on *OCT1* genotypes

OCT1 genotype explained 9% of variance in plasma concentrations of ondansetron

OCT1 genotype explained 1.8% of variance in frequency of vomiting and *CYP2D6* genotype explained 1.2%

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Fernandez-Rozadilla et al. 2013 Purpose: Conduct GWAS to determine genetic variations causing adverse drug reactions induced by 5-FU or FOLFOX in colorectal cancer patients Design: Prospective, cohort study CTX: High emetogenicity 5-FU – 560 patients FOLFOX – 457 patients Major outcome(s): Novel SNP discovery through GWAS analysis of patients who experienced emetic episode(s) during CTX treatment Novel SNP discovery through GWAS analysis of patients who	Exploratory analysis of SNPs in this study by GWAS SNPs identified in GWAS to be associated with CINV that were evaluated in Phase II 5-FU-CINV: rs10182133 rs2060645 rs6815391 rs7094179 rs9300811 Oxaliplatin-CINV: rs2389972 rs10158985 rs851974 rs2739171 rs724975 and Copy number variant on chromosome 2p22.3 (deletion) Protein function for SNPs not reported	N = 226 (Phase I) 5-FU = 93 FOLFOX =133 and 791 (Phase II) 5-FU = 467 FOLFOX = 324 Age: Mean (range) Phase I 5-FU: 72 (26-86) years FOLFOX: 69 (42-85) years Phase II 5-FU: 62 (21-83) years FOLFOX: 62 (26-75) years Gender: Phase I 5-FU: 43% male FOLFOX: 32% male Phase II 5-FU: 41% male FOLFOX: 43% male Diagnosis: Colorectal cancer (stage III or higher) = 100% Setting: Outpatient Antiemetic treatment: Not reported	Assessment of CINV: Nausea: Severity of nausea documented using WHO toxicity grading scale. Vomiting: Severity of vomiting documented using WHO toxicity grading scale Timing of CINV assessment: During CTX treatment. Specific time not reported Genotyping methods: SNP Microarray Affymetrix chip – Phase I Sequenom MassARRAY system – Phase II Statistical analyses: Logistic regression analysis to determine SNP association. Covariate adjustment was performed to correct for gender and	Phase I Of the 88 patients in Phase I who received 5-FU, 8 patients experienced CINV Of the 115 patients in Phase I who received FOLFOX, 23 patients experienced CINV None of the SNPs identified in Phase I reached the established genome wide significance level of 10E-07 Phase II Of the 467 patients in Phase II who received 5-FU, 96 patients experienced CINV Of the 341 patients in Phase II who received FOLFOX, 109 patients experienced CINV None of the SNPs tested in	Strengths: First GWAS to predict CINV associated with 5-FU and FOLFOX in patients with colorectal cancer Limitations: Nausea and vomiting were considered as a single phenotype Relatively small sample for a two - phase GWAS No report on whether the patients were on an antiemetic regimen Timing of CINV assessment was not clear

Major outcome(s):	Statistical analyses:	Major Findings:
experienced nausea during CTX treatment	severity of toxicities Odds ratio and 95% CI were calculated for each SNP to determine each SNPs association with 5-FU and FOLFOX induced toxicities Genome-wide significance level was set at p $\leq$ 10E-07	Phase II demonstrated significant associations with either 5-FU or FOLFOX induced CINV

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Tsuji et al. 2013 Purpose: Evaluate the association between the antiemetic efficacy of granisetron and dexamethasone and two polymorphisms in the *ABCB1* gene Design: Prospective, cohort study CTX: Doxorubicin + cyclophosphamide – 64 patients Major outcome(s): Association between genotype and complete response in acute and delayed CINV	Transporter protein *ABCB1* rs1045642 (3435 C>T) rs20325282 (2677 G>T/A)	N = 64 Age: 53.8 ± 9.8 years Gender: 100% female Diagnosis: Breast cancer = 100% Setting: Outpatient Antiemetic treatment: 20 mg of dexamethasone and either 3 mg (n = 33) or 1 mg (n = 31) of granisetron	Assessment of CINV: Nausea: Patient diary Vomiting: Patient diary Timing of CINV assessment: First 24 hours after CTX for acute phase and 4 days following CTX for delayed phase Genotyping methods: PCR-RFLP Statistical analyses: Cochran-Mantel-Haenzel test to determine the relationship between *ABCB1* polymorphisms and prophylactic antiemetic response to granisetron in combination with dexamethasone Logistic regression analysis to determine the effect of *ABCB1* genotype on the risk of acute and delayed CINV	For the 64 patients, frequency of *ABCB1* 2677 (rs20325282) GG, GT, GA, TT, TA and AA genotypes was 18.8%, 39.1%, 15.6%, 14.1%, 6.3% and 6.3% Frequency of *ABCB1* 3435 (rs1045642) CC, CT, and TT genotypes was 32.8%, 48.4%, and 18.8% Of the 64 patients, 64.1% had complete response for acute phase and 45.3% had complete response for delayed phase For *ABCB1* 2677 genotypes, complete response for acute phase was 83.3% for GG, 68.6% for GT and GA, and 41.2% for TT, TA, and AA carriers (p = 0.047)	Strengths: Exploratory study to determine the effect of *ABCB1* on acute and delayed CINV Patients received similar CTX and antiemetic treatment Limitations: Small sample size to determine the effect size of *ABCB1* genotype on acute and delayed CINV Unclear definition of nausea and vomiting

Major Findings:

Patients with *ABCB1* 2677 TT genotype were at an increased risk for acute CINV (OR, 17.500; 95% CI = 1.97 to 155.92, p = 0.045)

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: He et al. 2014 Purpose: Evaluate the association between the antiemetic efficacy of ondansetron and three polymorphisms in the *ABCB1* gene in Chinese AML patients treated with high dose of cytarabine CTX Design: Prospective, cohort study CTX: Cytarabine (1.5 g/m^2) – 215 patients Major outcome(s): Association between emetic response in first 1 to 5 days of CTX infusion and *ABCB1* genotype	Transporter protein *ABCB1* rs1045642 (3435 C>T) rs20325282 (2677 G>T/A) rs1128503 (1236 T>C)	N = 215 Age: 43.6 (mean) (range 14-57) years Gender: 47.9% female Diagnosis: Acute myeloid leukemia = 100% Setting: Outpatient Antiemetic treatment: 8 mg of ondansetron 30 minutes before CTX followed by 24 mg of ondansetron in a continuous infusion for 12 hours. 8 mg of ondansetron given once per day for 2 days after end of CTX to prevent delayed CINV	Assessment of CINV: Nausea: Patient diary and nausea VAS. A score >5 on the VAS was indicative of nausea Acute nausea divided into grades 1-2 and 3-4 based on the NCICTC v.3. Delayed nausea was categorized as a dichotomous variable (yes/no) Vomiting: Patient diary Acute vomiting divided into grades 1-2 and 3-4 based on the NCICTC v.3. Delayed vomiting was categorized as a dichotomous variable (yes/no) Timing of CINV assessment: Record daily occurrence of CINV from day 1 to day 5 following CTX Genotyping methods: Allele specific matrix-assisted laser desorption/ionization-time-of-	*ABCB1* 1236T>C was not in Hardy Weinberg equilibrium and its allelic frequency was not consistent with previous studies on Chinese Han population. This allele was not investigated further *ABCB1* 2677 G>T/A and 3435 C>T met Hardy Weinberg criteria and were evaluated Among the four haplotypes of the two SNPs, CG was the most predominant (48.3%) followed by TT/A (34.8%) Patients with CC genotype in *ABCB1* C3435T had a higher incidence of grade 3-4 acute CIN compared to patients with CT or TT genotype (p = 0.01). These findings were similar for patients who experienced	Strengths: First study to evaluate effect of *ABCB1* SNPs on CINV in Chinese Han population Relatively large sample size Conservative inclusion and exclusion criteria Patients received similar CTX and antiemetic treatment regimen Nausea and vomiting assessed simultaneously Limitations: *ABCB1* 1236T>C SNP could not be evaluated Methodology for validation and quality control of genotyping assay was not clear

Genotyping methods:	Major Findings:
flight mass spectrometry Statistical analyses: Logistic regression analysis to determine differences among groups after adjusting for age, gender, BMI, BSA, smoking, and drinking status	grade 3-4 acute CIV ($p = 0.002$) Patients with GG genotype in *ABCB1* 2677 G>T/A had a higher likelihood of experiencing grade 3-4 acute CIN than patients with GT/A or TT/A genotypes ($p = 0.012$) No association was found between polymorphisms in *ABCB1* genotype and the occurrence of delayed CINV Multivariate analysis indicated that patients who were female (OR = 0.214, 95% CI = 0.054 to 0.851, $p = 0.029$) and were CC homozygotes for *ABCB1* C3435T were at higher risk for acute CIV compared to male patients and carriers of CT and TT genotype In the multivariate analysis, the CC genotype for *ABCB1* C3435T was not significant for acute CIN

Major Findings:

Patients with the CG haplotype for *ABCB1* 3435 C>T and 2677 G>T/A had a higher likelihood of experiencing grade 3-4 acute CINV compared to other haplotypes of *ABCB1* (OR = 2.778, 95% CI = 1.416 to 5.451, p = 0.003 (for CIN); OR = 2.139, 95% CI = 1.040 to 4.401, p = 0.039 (for CIV))

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Lamba et al. 2014 Purpose: Identify SNPs in genes of relevance to the pharmacokinetic pharmacodynamic pathways of platinating agents and taxanes that are associated with outcomes and toxicity in patients with advanced NSCLC who were treated primarily with carboplatin-doublet CTX Design: Prospective, cohort study CTX: Moderate to high emetogenicity Carboplatin + paclitaxel – 77 patients Carboplatin + gemcitabine – 9 patients Carboplatin + etoposide – 2 patients Cisplatin + etoposide – 2 patients Major outcome(s): Association	Transporter proteins *ABCB1* rs1128503 *ABCC1* rs246240 rs2238476 *ABCG2* rs2231142 *ATP7B* rs1801244 Coiled coil domain protein *CCDC127* rs9312960 Drug metabolizing enzymes *CYP2C8* rs11572080 *NQO1* rs1800566 Nucleotide excision repair protein *ERCC4* rs744154 *XPC* rs2228001 Transcription factor *GTF2E1* rs447978	N = 90 Age: 66 (median) years Gender: 100% male Diagnosis: NSCLC stage IIIB = 19% NSCLC stage IV = 81% Setting: Outpatient Antiemetic treatment: Not reported	Assessment of CINV: Nausea: Self-report at the oncology clinic Nausea on the CTCAE v4 was operationalized as a categorical variable from grade 1 to 3 where 1 indicates loss of appetite and 3 indicates inadequate intake Vomiting: Self-report at the oncology clinic Vomiting on the CTCAE v4 was operationalized as a categorical variable from grade 1 to 5 where 1 indicates 1-2 episodes of emesis in 24 hours after CTX and 5 indicates death Timing of CINV assessment: Prior to first cycle of CTX administration and before each subsequent cycle Genotyping methods: Sequenome platform to	All genomic models were evaluated after controlling for age and number of CTX treatment cycles Nausea was associated with *ATP7B* rs1801244 missense mutation (OR (dominant model) = 4.63, 95% CI = 0.18 to 2.89, p = 0.027 and OR (additive model) = 1.93, 95% CI = -0.07 to 1.38, p = 0.078) Nausea was associated with *ABCG2* rs2231142 missense mutation (OR (dominant model) = 4.05, 95% CI = 0.03 to 2.77, p = 0.045 and OR (additive model) = 3.94, 95% CI = 0.03 to 2.71, p = 0.045) Nausea was associated with a SNP in the intronic region of a transcription factor *GTF2E1*	Strengths: Exploratory analysis to determine association between SNPs in candidate genes, that play a role in drug metabolizing pathways that were identified in a GWAS analysis Homogeneous sample of NSCLC patients with majority on the same CTX regimen Limitations: A very small sample size to determine the associations between a relatively large number of SNPs and occurrence of CINV For *ATP7B* and *ABCG2* while the p-values for findings associated with additive and dominant models for nausea were significant, the 95% CI included 1.0

Major outcome(s):	Gene(s) Classified by Function[+]:	Genotyping methods:	Major Findings:	Limitations:
between candidate gene variants implicated in CTX drug metabolism and CINV occurrence	Voltage gated ion channel *KCNC1* rs17718902 Motor protein *KLC3* rs13181 Integral membrane protein *TMEM63A* rs10158985 Tumor suppressor *TP53* rs1625895	genotype 63 SNPs in 29 genes Statistical analyses: Genetic models were coded as additive or dominant Cox proportional hazards model to determine the association between each SNP and nausea and vomiting	rs447978 (OR (dominant model) = 0.22, 95% CI = -2.52 to -0.49, p = 0.004 and OR (additive model) = 0.41, 95% CI = -1.67 to -0.12, p = 0.024) No SNPs were found to be associated with emesis	No report on whether the patients were on an antiemetic regimen Only 80 patients from a total of 635 had blood sample available for genomic analysis

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Lee et al. 2014 Purpose: Investigate associations between polymorphisms in *DPYD* gene and 5-FU toxicities in a large sample of patients with CRC Design: Prospective, cohort study CTX: Highly emetogenic FOLFOX only FOLFOX + cetuximab FOLFIRI only FOLFIRI + cetuximab or Six cycles of FOLFOX followed by six cycles of FOLFIRI + cetuximab Number of patients who received each treatment regimen not reported Major outcome(s): Association	While a total of 25 *DPYD* polymorphisms were genotyped, data on only 4 polymorphisms were reported Dihydropyrimidine dehydrogenase enzyme *DPYD* rs3918290 (*DPYD*2A*) Is a splice donor variant rs67376798 (D949V) rs55886062 (I560S) rs143986398 (P92A)	N = 2886 Age: 58 (median) (range 19-86) years Gender: 53.2% male Diagnosis: Colon cancer stage III = 100% Setting: Outpatient Antiemetic treatment: Not reported	Assessment of CINV: Nausea: Self report based on NCICTC Vomiting: Self report based on NCICTC Timing of CINV assessment: Biweekly Genotyping methods: Multiplex PCR amplification in combination with mass spectrometry on Sequenom MassARRAY system Statistical analyses: Patients with grade ≥3 nausea/vomiting were considered to have experienced toxicity. A total of 2594 patients had complete AE and genotype data Chi-square or Fisher's exact test, unequal variance two-sample *t* test,	Of the 2594 patients, 124 experienced CINV Older patients were more likely to experience 5-FU associated adverse events than younger patients ($p < 0.001$) Females reported higher 5-FU related adverse events compared to males ($p < 0.001$) Patients with the *DPYD*2A* (c.1905 + 1 G>A) splice donor variant were at an increased risk for 5-FU CINV ($p = 0.007$) 21 of the functionally deleterious *DPYD* polymorphisms were not present in the study sample	Strengths: Large sample size Conservative inclusion criteria Controlled for significant covariates in the statistical analysis Limitations: Separate phenotypic predictors of CINV were not reported No report on whether the patients were on an antiemetic regimen

Major outcome(s):	Statistical analyses:
between three well documented *DPYD* gene polymorphisms and the occurrence of CINV	and Wilcox rank sum test used to compare categorical variables, continuous variables, and counts with patients' *DPYD* status
	Logistic regression used to assess association between SNP status and occurrence of nausea and vomiting

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Pud et al. 2014 Purpose: Investigate association between genetic variations in *5-HT3C receptor, GCH1,* and *COMT* genes and chemotherapy induced symptoms in patients receiving adjuvant CTX for early breast cancer Design: Prospective, cohort study CTX: Highly emetogenic Cyclophosphamide + doxorubicin – 4 patients Cyclophosphamide + doxorubicin + paclitaxel – 75 patients Cyclophosphamide + doxorubicin + paclitaxel + trastuzumab – 30 patients Cyclophosphamide + doxorubicin + trastuzumab – 1 patient Major outcome(s): Association	Serotonin receptor *5-HT3RC* rs6766410 rs6807362 GTP cyclohydrolase I enzyme *GCH1* rs10483639 rs3783641 rs8007267 Catecholamine-*o*-methyltransferase enzyme *COMT* rs4818	N = 110 Age: 45.5 ± 10.1 years Gender: 100% female Diagnosis: Breast cancer stage I-IIIA = 100% Setting: Outpatient Antiemetic treatment: Not reported	Assessment of CINV: Nausea: Self-report – MSAS translated to Hebrew was used to determine severity scores of CIN Vomiting: Self-report – MSAS translated to Hebrew was used to determine severity scores of CIV Timing of CINV assessment: Once in 7 day period for each cycle of CTX Genotyping methods: PCR-RFLP Statistical analyses: One-way ANOVA to determine differences in CINV severity scores for *5-HT3C receptor* rs6766410 and for *5-HT3C receptor* rs6807362 polymorphisms respectively	For *5-HTRC3* rs6766410, a significant difference in the severity of CIN was found among the three genotypes: CC (0.8 ± 1.2), CA (1.5 ± 1.4) and AA (1.6 ± 1.6, p = 0.04) For *5-HTRC3* rs6766410, no differences in the severity of CIV were found among the three genotypes (i.e., CC, CA, AA) For *5-HTRC3* rs6807632, no differences in the severity of CIN or CIV were found among the three genotypes (i.e., GG, GC, CC) No associations were found between polymorphisms in *GCH1* or *COMT* and nausea and vomiting	Strengths: Relatively large sample size Conservative inclusion criteria Homogeneous sample of patients Limitations: Did not control for CINV risk factors such as history of alcohol consumption and history of CTX treatment Acute versus delayed CINV was not assessed No report on whether the patients were on an antiemetic regimen

Major outcome(s):

between functional variants in *5-HT3C receptor* and severity of nausea and vomiting

Author, Year Purpose, Study Design, Emetogenicity of CTX, Major outcome(s)	Gene(s) Classified by Function+	Sample Characteristics (sample size, age, gender, diagnosis, setting, antiemetic treatment)	Assessment of CINV, Timing of CINV Assessments, Genotyping Methods, Statistical Analyses	Major Findings	Strengths and Limitations
Author: Zoto et al. 2015 Purpose: Investigate the effect of genetic variants and haplotype of *ABCB1* on the antiemetic efficacy of 5-HT3 receptor antagonists Design: Prospective, cohort study CTX: Moderate to high emetogenicity Platinum based – 126 patients Adriamycin + cyclophosphamide – 113 patients Major outcome(s): Association between nausea and emetic occurrence in first 1 to 5 days of CTX infusion and *ABCB1* genotype Association between response to antiemetic treatment and *ABCB1* haplotypes	Transporter protein *ABCB1* rs1045642 (3435 C>T) rs20325282 (2677 G>T/A) rs1128503 (1236 T>C)	N = 239 Age: 51.2 ± 12.2 years Gender: 46% male Diagnosis: Gastrointestinal cancer = 31.4% Breast cancer = 27.6% Lymphoma = 15.1% Lung cancer = 13.4% Genitourinary cancer = 5.0% Other = 7.5% Setting: Outpatient Antiemetic treatment: Standardized regimen of either granisetron (64.9%) or ondansetron (35.1%) and dexamethasone (100%)	Assessment of CINV: Nausea: Self-report with daily questionnaire and severity rating as none, slight, moderate, or severe Vomiting: Self-report with daily questionnaire to record number of vomiting episodes Timing of CINV assessment: Daily questionnaires completed for five consecutive days from the start of CTX. Assessment evaluated for acute (0-24 hours) and delayed phases (25-120 hours) of CINV Genotyping methods: PCR-RFLP Statistical analyses: Nausea and vomiting were dichotomized as total control (i.e., absence of any degree of	In the acute phase, patients with *ABCB1* 3435 TT genotype (64.7%) had a higher control rate of CINV than patients with 3435 CT and 3435 CC genotypes (45.7%) (p = 0.016) In the acute phase, patients with *ABCB1* 1236 TT genotype (65.1%) had a higher control rate of CINV than patients with 1236 CT and 1236 CC genotypes (46.4%) (p = 0.027) In the acute phase, patients with *ABCB1* 2677 TT genotype (66.7%) had a higher control rate of CINV than patients with 2677 GG, 2677 GA and 2677 GT genotypes (46.5%) (p = 0.021)	Strengths: Relatively large sample size Conservative inclusion and exclusion criteria Limitations: It was not clear if the daily questionnaire was validated While data on the severity of CINV were collected, these data were analyzed as a categorical variable

Statistical analyses:	Major Findings:
nausea and absence of any degree of vomiting: yes/no) Mann-Whitney *U*-test used to measure differences in antiemetic response rate among *ABCB1* genotypes. Logistic regression to determine association between clinical factors, demographic factors, genotype and CINV	When all homozygous carriers of the variant alleles in *ABCB1* were combined (i.e., 3435 TT, 1236 TT, and 2677 TT) into one group, these patients had a higher rate of acute CINV control than patients with the other genotypes (67.7% versus 47.1%, p = 0.032) Patients with the TT-TT-TT for the *ABCB1* gene, as compared to other genotypes, had a higher acute CINV response when using granisetron (68.4% versus 44.1%, p = 0.048) but not ondansetron (66.7% versus 52.8%, p = 0.374) In the delayed phase, no significant change was found between complete control of CINV and genotypes Results of logistic regression analysis found that during the acute phase,

Major Findings:

total control of CINV was significantly increased by the absence of previous CINV ($p < 0.0001$) and the *ABCB1* 3435 TT genotype ($p = 0.021$), but not by gender ($p = 0.052$), age ($p = 0.071$), *ABCB1* 2677 TT genotype ($p = 0.069$) and *ABCB1*1236 TT genotype ($p = 0.069$)

Abbreviations: 5-FU = 5-fluorouracil, 5-HT = 5-hydroxytryptamine (human), 5-HT3A receptor = 5-hydroxytryptamine 3A receptor (human), 5-HT3B receptor = 5-hydroxytryptamine 3B receptor (human), 5-HT3C receptor = 5-hydroxytryptamine 3C receptor (human), 5-HT3RC = 5-hydroxytryptamine 3C receptor (human), 5-HT3D receptor = 5-hydroxytryptamine 3D receptor (human), 5-HT3E receptor = 5-hydroxytryptamine 3E receptor (human), ABCB1 = ATP binding cassette subfamily B member 1 (human), ABCC1 = ATP binding cassette subfamily C member 1 (human), ABCG2 = ATP binding cassette subfamily G member 2 (human), AE = adverse events, ATP7B = ATPase copper transporting beta (human), AML = acute myeloid leukemia, ANOVA = analysis of variance, BMI = body mass index, BSA = body surface area, C88R = cysteine88-to-arginine, CCDC127 = Coiled coil domain containing protein 127 (human), CI = confidence interval, CIN = chemotherapy induced nausea, CINV = chemotherapy-induced nausea vomiting, CIV = chemotherapy-induced vomiting, COMT = catechol-o-methyltransferase (human), CTCAE v4 = Common Terminology Criteria for Adverse Events version 4.0, CRC = colorectal cancer, CTX = chemotherapy, CYP2C8 = cytochrome P450 family 2 subfamily C member 8 (human), CYP2D6 = cytochrome P450 family 2 subfamily D member 6 (human), DNA = deoxyribonucleic acid, DPYD = dihydropyrimidine dehydrogenase (human), ERCC4 = excision repair cross-complementation group 4 (human), EM = extensive metabolizers, FE = fisher's exact, FOLFOX = fluorouracil oxaliplatin, g = gram, G401S = glycine401-to-serine, FOLFIRI = fluorouracil irinotecan, G465R = glycine465-to-arginine, GCH1 = guanosine triphosphate cyclohydrolase1 (human), GTF2E1 = general transcription factor IIE subunit 1 (human), GWAS = genome wide association studies, HEK293 = human embryonic kidney 293, IM = intermediate metabolizers, KCNC1 = potassium voltage gated channel subfamily C member 1 (human), KLC3 = kinesin light chain 3 (human), M420del = deletion of methionine420, MDR1 = multi-drug resistance 1 (human), m^2 = meter square, mg = milligram, mm = millimeter, MPP = 1-methyl-4-phenylpyridinium, MSAS = memorial symptom assessment scale, NCICTC = National Cancer Institute Common Toxicity Criteria, NQO1 = nicotinamide adenine dinucleotide phosphate quinone dehydrogenase 1 (human), NSCLC = non-small cell lung cancer, OCT1 = organic cation transporter 1 (human), OR = Odds ratio, PCR = polymerase chain reaction, PCR-RFLP – polymerase chain reaction – restriction fragment length polymorphism, PM = poor metabolizers, qPCR = quantitative polymerase chain reaction, R61C = arginine61-to-cysteine, SLC22A1 = solute carrier family 22 member 1 (human), SNP = single nucleotide polymorphism, TMEM63A = transmembrane protein 63A (human), TP53 = tumor protein p53 (human), UM = ultrarapid metabolizers, v = version, VAS = visual analog scale, vs = versus, WHO = World Health Organization, XPC = xeroderma pigmentosum, complementation group C (human)

+ Function of genes are reported based on description provided by the authors in the published paper.

Chapter 3:

Risk factors associated with chemotherapy-induced nausea and impact of nausea on quality of life outcomes

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Nausea in the Week Prior to the Next Cycle and Impact of Nausea on Quality of Life Outcomes. Journal of Pain and Symptom Management. 2018. doi: 10.1016/j.jpainsymman.2018.05.019.

ABSTRACT

Context: Despite current advances in antiemetic treatments, between 19% to 58% of oncology patients experience chemotherapy-induced nausea (CIN).

Objectives: Aims of this study were to determine the occurrence, severity, and distress of CIN and evaluate for differences in demographic and clinical characteristics, symptom severity, stress; and quality of life (QOL) outcomes between oncology patients who did and did not report CIN in the week prior to CTX. Demographic, clinical, symptom, and stress characteristics associated with CIN occurrence were determined.

Methods: Patients (n=1296) completed questionnaires that provided information on demographic and clinical characteristics, symptom severity, stress, and QOL. Univariate analyses were performed to evaluate for differences in demographic and clinical characteristics, symptom severity, stress, and QOL scores between the two patient groups. Multiple logistic regression analysis was used to evaluate for factors associated with nausea group membership.

Results: Of the 1296 patients, 47.5% reported CIN. In the CIN group, 15% rated CIN as severe and 23% reported high distress. Factors associated with CIN group membership included: less education; having childcare responsibilities; poorer functional status; higher levels of depression, sleep disturbance, evening fatigue, and intrusive thoughts; as well as receipt of CTX on a 14-day CTX cycle and receipt of an antiemetic regimen that contained serotonin receptor antagonist and steroid. Patients in the CIN group experienced clinically meaningful decrements in QOL.

Conclusions: This study identified new factors (e.g., poorer functional status, stress) associated with CIN occurrence. CIN negatively impacted patients' QOL. Pre-emptive and ongoing interventions may alleviate CIN occurrence in high risk patients.

Key words: nausea; chemotherapy; antiemetics; cancer; stress; quality of life

INTRODUCTION

With the advent of antiemetic prophylaxis, significant progress has been made in the prevention and treatment of chemotherapy-induced vomiting (CIV).(1) However, the management of chemotherapy-induced nausea (CIN) remains a significant clinical problem. While only 13% to 32% of patients report CIV, CIN occurs in 19% to 58% of oncology patients.(1) Unrelieved CIN can lead to compromised nutritional status, decrements in quality of life (QOL), and discontinuation of cancer treatment.(2)

A number of studies used multivariate logistic regression analysis to determine demographic and clinical characteristics associated with CIN.(2-9) In terms of demographic characteristics, findings are not consistent. While in three studies,(2-4) age <50 years was associated with increased risk for CIN, in two studies,(5, 6) no age association was found. Similarly, while in three studies,(2, 3, 5) female patients were more likely to report CIN, in three studies,(2, 6, 7) no association was reported.

In terms of clinical characteristics, the most common risk factors for CIN include: a history of motion sickness,(1, 6, 8) a history of morning sickness,(1, 8, 10) malnutrition,(11, 12) and a history of nausea and emesis.(1, 9) In addition, the intrinsic emetogenic potential of the chemotherapy (CTX) regimen contributes to the occurrence of CIN.(2, 4) While in one study,(2) decreased alcohol intake was shown to increase the risk for CIN, this association was not supported in other studies.(8, 9)

Across six studies, pre-CTX nausea,(2, 9) pre-CTX anxiety,(2, 8, 9, 13) less than seven hours of sleep on the night before CTX,(8) as well as higher levels of depression,(5) and fatigue (12, 13) post-CTX were associated with the occurrence and severity of CIN. While most of the studies that evaluated for predictors of CIN had relatively large sample sizes, inconsistent findings may be related to: the variety of instruments used to assess nausea;(3, 5, 6, 8, 11) lack of

controls for ethnicity in the multivariate analyses;(2, 3, 5, 8) and diverse factors evaluated across these studies.(3, 6, 8)

While previous studies provide insights into risk factors for CIN, additional research is warranted. First, additional demographic and clinical characteristics associated with other common symptoms in oncology patients (e.g., ethnicity,(14) education,(5, 15) adult/child care responsibilities,(16) functional status,(15, 16) body mass index,(11, 12) comorbidities,(16) and treatment-related factors (5)) need to be evaluated. Second, the intrinsic emetogenic potential of the CTX regimen and the type of antiemetic regimen patients received need to be included in a multivariate analysis. Finally, the impact of concurrent symptoms on the occurrence of CIN warrants investigation.

The stress associated with cancer and its treatment can lead to symptoms such as depression and anxiety.(17) In a recent study of the effect of an integrated yoga program on CIN and CIV,(18) the authors suggested that the positive effect of the intervention on these two symptoms was through a decrease in stress. However, no studies have evaluated for associations between perceived stress and the occurrence of CIN.

While the impact of cancer symptoms on QOL outcomes continues to be an area of active investigation,(19-21) the majority of the studies on the associations between CTX-induced nausea and vomiting (CINV) and QOL did not distinguish between CIN and CIV and/or were done in the context of clinical trials of antiemetics.(22-24) In addition, most studies used a global measure of QOL and did not evaluate for associations between the occurrence of CIN and various domains of QOL (e.g., physical or social well-being).

Therefore, in a sample of oncology patients receiving CTX (n=1296), the purposes of this study were to evaluate for the occurrence, severity, and distress of CIN and evaluate for differences in demographic and clinical characteristics, symptom severity, perceived stress, and

QOL outcomes between patients who did and did not report CIN in the week prior to their next dose of CTX. In addition, we determined which demographic, clinical, symptom, and stress characteristics were associated with the occurrence of nausea.

METHODS

Patients and settings

This study analyzed data collected as part of a larger descriptive, longitudinal study that evaluated the symptom experience of oncology outpatients receiving CTX.(25, 26) Patients were included if they: were ≥18 years of age; had a diagnosis of breast, gastrointestinal, gynecological, or lung cancer; had received CTX within the preceding four weeks; were scheduled to receive at least two additional cycles of CTX; were able to read, write, and understand English; and provided written informed consent. Patients were recruited from two Comprehensive Cancer Centers, one Veteran's Affairs hospital, and four community-based oncology programs. The study was approved by the Committee on Human Research at the University of California at San Francisco and by the Institutional Review Board at each of the study sites.

Study procedures

A total of 2234 patients were approached and 1343 consented to participate (60.1% response rate). The major reason for refusal was being overwhelmed with their cancer treatment. A research staff member approached eligible patients in the infusion unit and discussed participation in the study. Written informed consent was obtained from all of the patients. Because of the stress associated with the first treatment, patients were recruited during their second or the third cycle of CTX. Depending on the length of their CTX cycle (i.e., 14-day, 21-day, or 28-day), patients completed all of the study questionnaires in their homes, a total of six times over the two cycles of CTX. The enrollment assessment (i.e., the assessment of nausea in

the week prior to the patients' next cycle of CTX) was used in this analysis to create the nausea groups. Medical records were reviewed for disease and treatment information.

Instruments

Demographic and clinical characteristics - Demographic questionnaire obtained information on: age, gender, ethnicity, marital status, living arrangements, education, employment status, income, and past medical history. Karnofsky Performance Status (KPS) scale was used to evaluate functional status.(27) Self-Administered Comorbidity Questionnaire (SCQ) evaluated the occurrence, treatment, and functional impact of thirteen common comorbid conditions.(28) Total SCQ score ranges from 0 to 39. Alcohol Use Disorders Identification Test (AUDIT) evaluated alcohol consumption, alcohol dependence, and the consequences of alcohol abuse in the last 12 months.(29) Smoking questionnaire assessed smoking history.(30)

Assessment of nausea - Memorial Symptom Assessment Scale (MSAS) was used to assess nausea. Patients were asked to indicate whether or not they had experienced nausea in the past week (i.e., symptom occurrence). If they experienced nausea, they were asked to rate its frequency, severity, and distress.(31) Patients' assessment of nausea in the week prior to their next cycle of CTX (i.e., enrollment assessment) was used to dichotomize the sample. Patients who provided a rating for occurrence, frequency, severity, and/or distress for the nausea item were coded as having nausea. Patients who indicated "no" to the occurrence item were coded as not having nausea.

Assessment of other symptoms - Associations between the occurrence of nausea and other common symptoms were evaluated using a number of valid and reliable instruments. Diurnal variations in fatigue and decrements in energy were evaluated using the Lee Fatigue Scale (LFS).(32) State and trait anxiety were evaluated using the Spielberger State-Trait Anxiety Inventories.(33) Depressive symptoms were assessed using the Center for Epidemiological

Studies-Depression scale (CES-D).(34) The quality of sleep was evaluated using the General Sleep Disturbance Scale (GSDS).(35) Difficulties with executive function were assessed using the Attentional Function Index (AFI).(36) Occurrence of pain was evaluated using the Brief Pain Inventory.(37)

Assessment of stress – Stress was assessed using disease-specific (i.e., Impact of Event Scale-Revised (IES-R) (38)) and general (i.e., Perceived Stress Scale (PSS) (39)) stress measures. Three subscales in the IES-R evaluate the level of intrusion, avoidance, and hyperarousal associated with cancer and its treatment.(40) PSS evaluates stress due to life circumstances. For both instruments, a higher score indicates greater stress.(39)

Assessment of QOL - QOL was evaluated using disease-specific (i.e., QOL-Patient Version (QOL-PV) (41)) and generic (i.e., Medical Outcomes Study-Short Form-12 (SF-12) (42)) measures. The QOL-PV assesses four domains of QOL (i.e., physical, psychological, social, and spiritual well-being) as well as a total QOL score. Higher scores indicate a better QOL.(41) The SF-12 consists of 12 questions about physical and mental health as well as overall health status. The SF-12 is scored into: physical component summary (PCS) and mental component summary (MCS) scores. Higher summary scores indicate a better QOL.(42)

Coding of the emetogenicity of the CTX regimens

Using the Multinational Association for Supportive Care in Cancer (MASCC) guidelines (43), each CTX drug in the regimen was classified as having: minimal, low, moderate, or high emetogenic potential. The emetogenicity of the regimen was categorized into one of three groups (i.e., low/minimal, moderate, or high) based on the CTX drug with highest emetogenic potential.

An exception was made if a patient received doxorubicin and cyclophosphamide. When administered separately, doxorubicin and cyclophosphamide are listed as having moderate emetogenic potential (43). When given together, the combination has high emetogenic potential.

Coding of the antiemetic regimens

Each antiemetic was coded as either a neurokinin-1 (NK-1) receptor antagonist, a serotonin receptor antagonist, a dopamine receptor antagonist, prochlorperazine, lorazepam, or a steroid. The antiemetic regimens were coded into one of four groups: none (i.e., no antiemetics administered); steroid alone or serotonin receptor antagonist alone; serotonin receptor antagonist and steroid; or NK-1 receptor antagonist and two other antiemetics (e.g., a serotonin receptor antagonist, dopamine receptor antagonist, prochlorperazine, lorazepam and/or a steroid).

Statistical analyses

Data were analyzed using SPSS Version 23 (IBM, Armonk, NY). Descriptive statistics and frequency distributions were calculated for demographic and clinical characteristics. For categorical variables, nonparametric tests were used to evaluate for differences in demographic and clinical characteristics between patients who did and did not report CIN. For continuous variables, Independent Student's t-tests were done to evaluate for differences in demographic and clinical characteristics, as well as symptom severity, perceived stress, and QOL scores between patients who did and did not report CIN. Spearman's correlation was used to evaluate the relationships between the categorical variables. Effect sizes were determined using Cohen's d statistic.(44)

Multiple logistic regression analysis was used to evaluate for predictors of nausea group membership. Only those characteristics that were significantly different in the univariate analyses between patients who did and did not report CIN were evaluated in the logistic regression analysis. A backwards stepwise approach was used to create a parsimonious model. Only predictors with a p-value of <0.05 were retained in the final model.

RESULTS

Nausea characteristics

Of the 1296 patients who responded to the nausea item, 615 (47.5%) reported nausea in the week prior to their next cycle of CTX. Of the 615 patients who reported nausea, 95.3% (n=586) rated its severity. As illustrated in Figure 1A, 11% (n=66) of the patients reported "severe" and 4% (n=25) reported "very severe" nausea. Of the 615 patients who reported nausea, 95.0% (n=548) rated its distress. As illustrated in Figure 1B, 14% (n=80) of the patients reported "quite a bit" and 9% (n=50) reported "very much" distress related to nausea.

Differences in demographic and clinical characteristics

Compared to the no nausea group, patients who reported nausea were significantly younger and less educated; had a lower KPS score, and had an increased number of comorbidities, a higher comorbidity score, and a lower AUDIT score. A higher percentage of patients in the nausea group reported child care responsibilities, had a lower annual income, and were less likely to be employed (Table 1).

Patients in the nausea group were more likely to have diabetes, anemia or blood disease, depression, and back pain. In terms of cycle length, a higher percentage of patients in the nausea group received CTX on a 14-day cycle compared to those in the no nausea group. A lower percentage of patients in the nausea group received CTX on a 21-day cycle compared to those in the no nausea group. In terms of emetogenicity of the regimen, a higher percentage of patients in the nausea group received highly emetogenic CTX. In terms of the antiemetic regimen, while a lower percentage of patients in the nausea group received a steroid alone or serotonin receptor antagonist alone compared to the no nausea group, a higher percentage of these patients received a NK-1 receptor antagonist and two other antiemetics compared to the no nausea group.

Differences in symptom severity

Compared to the no nausea group, patients who reported nausea had significantly higher depression, trait anxiety, state anxiety, sleep disturbance, morning and evening fatigue scores and lower attentional function, morning, and evening energy scores. A significantly higher percentage of patients in the nausea group reported pain (Table 2).

Differences in perceived stress scores

Compared to the no nausea group, patients who reported nausea had a significantly higher PSS score. Patients in the nausea group reported significantly higher IES-R subscale (i.e., intrusion, avoidance and, hyperarousal) and total scores (Table 3).

Differences in QOL outcomes

Compared to the no nausea group, patients who reported nausea scored significantly lower on three QOL-PV subscales (i.e., physical, psychological, social well-being) as well as on the total score. For the SF-12, compared to the no nausea group, patients who reported nausea had significantly lower MCS and PCS scores (Table 4).

Logistic regression analysis of factors associated with nausea group membership

In the logistic regression analysis to determine factors associated with nausea group membership, characteristics that were significantly different between the two nausea groups in the univariate analysis ($p<0.05$) were included in the backwards stepwise elimination model (i.e., age, education, KPS score, SCQ score, child care responsibilities, employment status, CTX cycle length, antiemetic regimen, all of the symptom scores, PSS total score, and the three IES-R subscale scores).

While AUDIT score and income were significantly different between the two groups, 456 patients did not complete the AUDIT and 138 patients did not report their income. Therefore, these two variables were not included in the regression analysis. Consequently, data from 1035

patients were included in the final model. The inter-correlations among the potential predictors were examined for possible multicolinearity. Because trait anxiety and state anxiety scores were highly correlated (r = .82), only trait anxiety was evaluated in the initial model.

Ten variables were retained in the final logistic regression model (Table 5). Those variables were education, child care responsibilities, KPS score, CES-D score, GSDS score, evening LFS score, PSS total score, IES-R intrusion subscale score, CTX cycle length, and antiemetic regimen. The overall model was significant ($X^2 = 189.99$, $p<0.001$). Patients who were less educated; had child care responsibilities; had a lower KPS score; had higher depression, sleep disturbance, evening fatigue, and IES-R intrusion scores; and had a lower PSS score were more likely to be in the nausea group.

CTX cycle length and antiemetic regimen groups were significant predictors of nausea group membership. Because CTX cycle length had three groups, three pairwise contrasts were examined to interpret the effect of cycle length. The significance criteria for each of the contrasts was 0.0125 (0.05/3). Only one contrast was significant. Compared to patients who received a 14-day cycle, patients who received a 21-day cycle of CTX had a 42% decrease in the odds of belonging to the nausea group. Because antiemetic regimen had four groups, six pairwise contrasts were examined to interpret the effect of antiemetic regimen. The significance criteria for each of the contrasts was 0.0083 (0.05/6). Only one contrast was significant. Compared to patients who received a steroid alone or a serotonin receptor antagonist alone, patients who received a serotonin receptor antagonist and steroid were 1.73 times more likely to be in the nausea group.

In the final regression model, the emetogenicity of the CTX regimen was not a significant predictor of CIN. A number of additional analyses were done to explore this unexpected finding. First, antiemetic regimen and emetogenicity of the CTX regimen were moderately correlated

with each other ($r = 0.50$, $p<0.001$). Second, within the regression analysis, we tested for an interaction between emetogenicity of the CTX regimen and the antiemetic regimen. The interaction term was not significant. Third, we did another analysis where we removed cycle length from the analysis and forced emetogenicity of the CTX regimen into the regression analysis. Emetogenicity of the CTX was not a significant predictor of CIN group membership in this analysis ($p=0.33$).

DISCUSSION

This study is the first to evaluate the relative contribution of a comprehensive set of demographic and clinical characteristics, as well as symptom severity scores, and levels of perceived stress to the occurrence of nausea in the week prior to the patients' next cycle of CTX. In addition, this study is the first to evaluate multiple domains of QOL in patients who did and did not report CIN.

Given previous occurrence rates of 19% (1, 45) to 58%, (1, 46), our 47.5% occurrence rate is quite high. Consistent with a previous report,(11) 15% of our patients reported that the severity of CIN was severe and 23% reported high levels of distress. These findings suggest that unrelieved CIN continues to be a significant problem during CTX.

The results of the logistic regression analysis provide new insights into modifiable and nonmodifiable risk factors for CIN. While in the univariate analysis and consistent with previous studies, younger age (2, 3, 8, 47) and decreased alcohol intake (2) were associated with CIN, only education and having child care responsibilities remained significant in the multivariate model. Given that one study found no association with education and CIN,(5) additional research is needed to confirm our association. Our study is the first to report that patients who had child care responsibilities were 1.4 times more likely to be in the CIN group. Clinicians can assess whether patients need assistance with child care and make appropriate referrals.

While not evaluated in previous studies, in the univariate analysis, both a higher comorbidity burden and lower functional status were associated with CIN group membership. However, in the multivariate analysis, only KPS score was retained in the final model. The differences in KPS scores between the CIN and no CIN groups represent not only statistically significant, but clinically meaningful differences (i.e., Cohen's d = 0.60). While no studies evaluated for associations between functional status and CIN, previous studies found associations between lower KPS scores and higher depression,(48) anxiety,(49) fatigue,(16, 25) and sleep disturbance (15) scores.

This study is the first to evaluate for associations between CTX cycle length and CIN group membership. Compared to patients on the 21-day cycle, patients on a 14-day cycle were more likely to report nausea in the week prior to their next does of CTX. This association can partially be explained by the increased frequency of exposure to CTX. In addition, compared to patients on a 21-day cycle, a higher percentage of patients on a 14-day cycle received highly emetogenic CTX (36.8% vs 63.2%, $p<0.001$, respectively). While in our univariate analysis and consistent with previous studies,(2, 4, 9) the emetogenicity of the CTX regimen was associated with CIN group membership, only CTX cycle length and antiemetic regimen remained significant in our multivariate model. One of the most likely reason why all three characteristics did not remain significant in the multivariate analysis is that the emetogenicity of CTX regimen and antiemetic regimen were correlated ($r = 0.50$, $p = <0.001$). Another plausible explanation for this finding is that different factors may be associated with different CINV outcomes (e.g., occurrence of CIV, severity of CIN, severity of CIV).

In our multivariate model, compared to patients who received either a steroid or a serotonin receptor antagonist, patients who received the combination were more likely to belong to nausea group. While one would expect the opposite association, one possible explanation for

this finding is that compared to patients who received the single agent (10.2%), 89.8% of patients who received the combination antiemetic regimen received highly emetogenic CTX ($p<0.001$). Another factor that could explain this finding is patients' level of adherence with the antiemetic regimen. While not assessed in this study, future studies of CIN need to include a measure of antiemetic adherence as a covariate.

This study is the first to evaluate for associations between the severity of the most common symptoms reported by oncology patients and CIN group membership. For patients in the CIN group, all of the symptom severity scores were above the clinically meaningful cutoff scores. The findings in our regression analysis are consistent with previous reports that found associations between pre- and post-treatment CIN and higher levels of depression,(5) fatigue,(13) and sleep disturbance (8).

While previous studies found an association between CIN and higher levels of anxiety,(8, 9) trait anxiety scores did not remain significant in our multivariate model. This finding may be partially explained by the inclusion of stress scores in our predictive model. Our study is the first to evaluate for associations between CIN and measures of both disease specific and general stress. While all of the subscale and total IES-R scores for patients in the CIN group were significantly higher, the total IES-R score did not exceed the clinically meaningful IES-R cutoff score of $\geq$33.(38) In the multivariate analysis, for each 1 point increase on the intrusion subscale score, there was a 1.35 increased odds of being in the nausea group. The intrusion subscale assesses intrusive thoughts about the stress associated with cancer and its treatment (e.g., disturbing visuals and feelings). In cancer patients, fear of recurrence and progression of cancer, as well as physical symptoms (e.g., pain) are associated with increased stress.(50)

The PSS was used to evaluate association between non-specific stress that exceeds a person's coping abilities (39) and CIN. In the multivariate analysis, for each 1 point increase in

PSS score, there was a 3% decrease in odds of belonging to nausea group. This unexpected finding warrants evaluation in future studies.

Patients who reported CIN had not only statistically significant but clinically meaningful (i.e., Cohen's d = 0.45 to 0.81) decrements in overall QOL as well as in the physical, psychosocial, and social domains.(51) In addition, these patients had clinically meaningful (i.e., Cohen's d = 0.44 to 0.45) decrements in MCS and PCS scores.(44) Patients who reported CIN had a mean MCS score of 46.55 which is below the score of 50 for the general US population. While patients in the CIN group had lower PCS scores, both groups of patients had PCS scores that were below the normative value of 50. Our findings are consistent with previous studies that reported that higher symptom occurrence rates (e.g., fatigue,(52-54) pain,(52-54) sleep disturbance (52-54)) were associated with lower PCS and MCS scores. Clinicians need to educate patients about the importance of taking antiemetic medication as prescribed to decrease CIN and associated decrements in QOL.

Several limitations warrant consideration. In a previous study the occurrence of CIN during the first cycle of CTX was a risk factor for future episodes of CIN.(2) Because patients were enrolled during their second and third cycle of CTX, we could not assess the contribution of this risk factor or patients' expectations for CIN, to CIN group membership. In addition, we did not assess patients' level of adherence with their antiemetic regimen. While we did evaluate a large number of previously reported risk factors, because our study was not designed specifically to study CIN, a number of risk factors (e.g., morning sickness, motion sickness) were not assessed. Because of the cross-sectional nature of this study, longitudinal studies are needed to demonstrate causal relationships between our identified risk factors and changes over time in the occurrence of CIN.

Despite the limitations, our findings suggest that CIN occurs in a high percentage of oncology patients receiving CTX. The modifiable risk factors that were identified include: having childcare responsibilities; poorer functional status; and higher levels of depression, sleep disturbance, evening fatigue, perceived stress, and intrusive thoughts and feelings. Clinicians need to assess patients for these risk factors and refer them for appropriate interventions (e.g., physical therapy, mental health services). Clinicians need to educate patients about stress reduction strategies and the importance of adhering with the antiemetic regimen.

Future studies to evaluate risk factors for CIN should enroll CTX naïve patients and use instruments specifically designed to measure CIN occurrence and severity (e.g. MASCC Antiemesis Tool,(55) Morrow Assessment of Nausea and Emesis Follow-Up (56)). The use of these measures would provide a comprehensive evaluation of anticipatory, acute, and delayed nausea, as well as the effectiveness of the antiemetic regimen. Patient adherence with the antiemetic regimen needs to be evaluated to determine its association with CIN occurrence, severity and distress. Predictors identified in previous studies as well as those identified in our study warrant confirmation. Longitudinal studies of CIN occurrence may provide insights into which characteristics identify higher risk patients. Because severe nausea can have a negative impact on patients' nutritional status and physical functioning,(11) future studies need to examine these relationships over multiple cycles of CTX. This knowledge will assist clinicians to recommend more targeted interventions to decrease the occurrence and severity of CIN.

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Figure 3.1– Percentage of patients who reported each severity (A) and distress (B) rating for nausea on the Memorial Symptom Assessment Scale

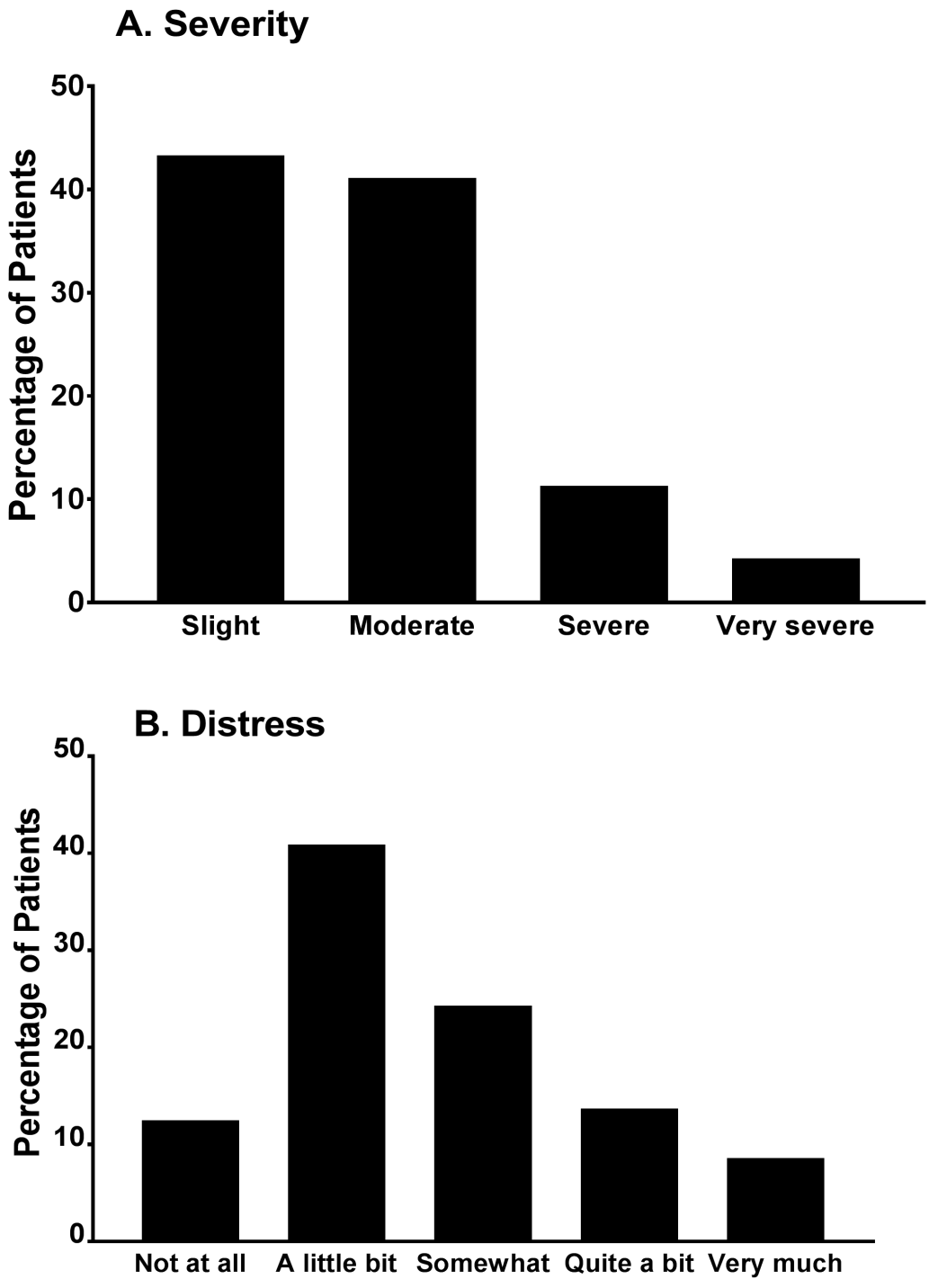

Table 3.1 – Differences in Demographic and Clinical Characteristics Between Patients With and Without Chemotherapy-Induced Nausea

Characteristic	No Nausea 52.5% (n=681)	Nausea 47.5% (n=615)	Statistics
	Mean (SD)	Mean (SD)	
Age (years)	58.64 (12.58)	55.62 (11.93)	t = 4.41, p < 0.001
Education (years)	16.43 (2.97)	15.87 (3.04)	t = 3.34, p = 0.001
Body mass index (kg/m^2)	26.15 (5.37)	26.36 (6.02)	t = -0.64, p = 0.520
Karnofsky Performance Status score	83.36 (11.54)	76.20 (12.41)	t = 10.50, p < 0.001
Number of comorbidities	2.30 (1.37)	2.53 (1.50)	t = -2.82, p = 0.005
SCQ score	5.14 (2.90)	5.87 (3.48)	t = -4.10, p < 0.001
AUDIT score	3.17 (2.52)	2.76 (2.44)	t = 2.39, p = 0.017
Time since cancer diagnosis (years)	2.07 (3.99)	1.79 (3.61)	U, p = 0.230
Time since diagnosis (median)	0.44	0.41	
Number of prior cancer treatments	0.77 (0.42)	0.73 (0.44)	t = 1.50, p = 0.132
Number of metastatic sites including lymph node involvement	1.28 (1.21)	1.18 (1.22)	t = 1.43, p = 0.153
Number of metastatic sites excluding lymph node involvement	0.81 (1.03)	0.73 (1.04)	t = 1.32, p = 0.188
	% (n)	% (n)	
Gender*			FE, p = 0.257
Female	76.2 (519)	79.0 (486)	
Male	23.6 (161)	21.0 (129)	
Transgender	0.2 (1)	0.0 (0)	
Ethnicity			X^2 = 5.57, p = 0.135
White	72.8 (490)	67.1 (407)	
Black	6.4 (43)	8.1 (49)	
Asian or Pacific Islander	11.4 (77)	12.7 (77)	
Hispanic Mixed or Other	9.4 (63)	12.2 (74)	
Married or partnered (% yes)	64.6 (435)	64.0 (388)	FE, p = 0.861
Lives alone (% yes)	21.6 (145)	21.9 (133)	FE, p = 0.946
Child care responsibilities (% yes)	18.5 (124)	26.2 (157)	FE, p = 0.001
Care of adult responsibilities (% yes)	7.1 (44)	8.7 (48)	FE, p = 0.328
Born prematurely (% yes)	4.4 (29)	6.4 (37)	FE, p = 0.163
Currently employed (% yes)	37.8 (255)	32.4 (197)	FE, p = 0.047
Income			KW, p < 0.001
< $30,000	12.5 (75)	25.0 (139)	
$30,000 to < $70,000	22.1 (133)	19.7 (110)	
$70,000 to < $100,000	17.0 (102)	16.9 (94)	
≥ $100,000	48.4 (291)	38.4 (214)	
Specific comorbidities (% yes)			
Heart disease	6.9 (47)	4.6 (28)	FE, p = 0.075
High blood pressure	31.1 (212)	29.6 (182)	FE, p = 0.586
Lung disease	11.2 (76)	11.5 (71)	FE, p = 0.861
Diabetes	7.2 (49)	10.9 (67)	FE, p = 0.025
Ulcer or stomach disease	3.8 (26)	6.0 (37)	FE, p = 0.071
Kidney disease	1.5 (10)	1.5 (9)	FE, p = 1.000
Liver disease	6.0 (41)	6.8 (42)	FE, p = 0.572
Anemia or blood disease	10.4 (71)	15.0 (92)	FE, p = 0.015
Depression	15.1 (103)	23.7 (146)	FE, p < 0.001
Osteoarthritis	12.5 (85)	11.7 (72)	FE, p = 0.733
Back pain	21.9 (149)	29.6 (182)	FE, p = 0.002
Rheumatoid arthritis	3.8 (26)	2.6 (16)	FE, p = 0.272
Exercise on a regular basis (% yes)	73.4 (493)	68.5 (408)	FE, p = 0.063

Characteristic	No Nausea 52.5% (n=681)	Nausea 47.5% (n=615)	Statistics
	% (n)	% (n)	
Smoking current or history of (% yes)	36.3 (244)	34.5 (208)	FE, p = 0.520
Cancer diagnosis			X^2 = 5.46, p = 0.141
Breast	40.5 (276)	39.5 (243)	
Gastrointestinal	28.5 (194)	33.3 (205)	
Gynecological	19.2 (131)	15.3 (94)	
Lung	11.7 (80)	11.9 (73)	
Type of prior cancer treatment			X^2 = 4.73, p = 0.193
No prior treatment	23.4 (155)	26.9 (161)	
Only surgery, CTX, or RT	42.7 (238)	41.6 (249)	
Surgery & CTX, or Surgery & RT, or CTX & RT	21.7 (144)	17.7 (106)	
Surgery & CTX & RT	12.2 (81)	13.7 (82)	
CTX cycle length			X^2 =17.77, p< 0.001
14 day cycle	37.2 (253)	48.3 (297)	0 < 1
21 day cycle	56.2 (382)	44.7 (275)	0 > 1
28 day cycle	6.6 (45)	7.0 (43)	NS
Emetogenicity of CTX			X^2 =14.88, p= 0.001
Minimal/Low	21.4 (146)	15.9 (98)	0 > 1
Moderate	62.6 (426)	60.5 (372)	NS
High	16.0 (109)	23.6 (145)	0 < 1
Antiemetic regimens			X^2 =19.82, p <0.001
None	8.2 (56)	5.9 (36)	NS
Steroid alone or serotonin receptor antagonist alone	24.1 (164)	16.4 (101)	0 > 1
Serotonin receptor antagonist and steroid	46.5 (317)	48.9 (301)	NS
NK-1 receptor antagonist and two other antiemetics	21.1 (144)	28.8 (177)	0 < 1

Abbreviations: AUDIT = Alcohol Use Disorders Identification Test, CTX = chemotherapy, FE = Fisher's Exact test, kg = kilograms, KW = Kruskal Wallis test, m^2 = 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^2 = Chi square

*Chi Square test done without the transgender participant

Table 3.2 - Differences in Symptom Severity Scores Between Patients With and Without Chemotherapy-Induced Nausea

Symptom	Clinically Meaningful Cut-off Scores	No Nausea 52.5% (n = 681) Mean (SD)	Nausea 47.5% (n = 615) Mean (SD)	Statistics
CES-D score	≥16.0	10.29 (8.56)	15.65 (10.14)	t = -10.08, p < 0.001
Trait Anxiety Inventory score	≥32.2	33.06 (9.82)	37.32 (10.69)	t = -7.34, p < 0.001
State Anxiety Inventory score	≥31.8	31.23 (11.07)	36.66 (13.19)	t = -7.88, p < 0.001
Attentional Function Index score	<5 Low 5 - 7.5 Moderate >7.5 High	6.81 (1.70)	5.95 (1.80)	t = 8.76, p < 0.001
General Sleep Disturbance Scale	≥43.0	46.82 (19.19)	58.50 (19.46)	t = -10.68, p < 0.001
Morning fatigue score (LFS)	≥3.2	2.48 (2.00)	3.80 (2.30)	t = -10.85, p < 0.001
Evening fatigue score (LFS)	≥5.6	4.89 (2.14)	5.81 (2.05)	t = -7.80, p < 0.001
Morning energy score (LFS)	≤6.2	4.64 (2.29)	4.14 (2.18)	t = 3.98, p < 0.001
Evening energy score (LFS)	≤3.5	3.68 (1.96)	3.40 (2.11)	t = 2.45, p = 0.015
Percentage of patients with pain (%, n)		49.3 (332)	64.8 (396)	FE, p < 0.001

Abbreviations: CES-D = Center for Epidemiological Studies-Depression Scale, FE = Fisher's Exact, LFS = Lee Fatigue Scale, SD = standard deviation

Table 3.3 - Differences in Stress Scores Between Patients With and Without Chemotherapy-Induced Nausea

Instrument	No Nausea 52.5% (n = 681)	Nausea 47.5% (n = 615)	Statistics
	Mean (SD)	Mean (SD)	
Perceived Stress Scale score	17.00 (7.86)	20.07 (8.30)	$t = -6.71$, $p < 0.001$
IES-R subscale scores			
Intrusion	0.76 (0.63)	1.07 (0.75)	$t = -7.82$, $p < 0.001$
Avoidance	0.86 (0.66)	1.05 (0.68)	$t = -5.08$, $p < 0.001$
Hyperarousal	0.52 (0.58)	0.81 (0.72)	$t = -7.86$, $p < 0.001$
IES-R total score	16.00 (11.75)	21.83 (13.84)	$t = -7.95$, $p < 0.001$

Abbreviations: IES-R = Impact of Event Scale-Revised, SD = standard deviation

Table 3.4 - Differences in Quality of Life Outcomes Between Patients With and Without Chemotherapy-Induced Nausea

Instrument	No Nausea 52.5% (n = 681)	Nausea 47.5% (n = 615)	Statistics
	Mean (SD)	Mean (SD)	
Quality of Life Scale - Patient Version			
Physical well-being	7.31 (1.54)	5.86 (1.76)	$t = 15.55$, $p < 0.001$
Psychological well-being	5.88 (1.79)	5.05 (1.85)	$t = 7.99$, $p < 0.001$
Social well-being	6.21 (1.90)	5.20 (2.01)	$t = 9.06$, $p < 0.001$
Spiritual well-being	5.38 (2.13)	5.57 (2.01)	$t = -1.66$, $p = 0.097$
Total score	6.13 (1.36)	5.33 (1.42)	$t = 10.13$, $p < 0.001$
Short Form12 Health Survey			
MCS score	51.21 (9.73)	46.55 (10.72)	$t = 7.85$, $p < 0.001$
PCS score	43.51 (10.08)	38.73 (10.50)	$t = 8.04$, $p < 0.001$

Abbreviations: MCS = mental component summary, PCS = physical component summary, SD = standard deviation

Table 3.5 - Multiple Logistic Regression Analysis Predicting Nausea Group Membership (n = 1035)

Predictor	Odds Ratio	95% CI	p-value
Education (years)	0.93	0.89, 0.98	0.003
Child care responsibilities	1.42	1.03, 1.97	0.032
Karnofsky Performance Status score	0.96	0.95, 0.98	< 0.001
CES-D score	1.03	1.00,1.05	0.026
General Sleep Disturbance Scale score	1.01	1.00,1.02	0.011
Evening fatigue score (LFS)	1.12	1.04,1.20	0.003
Perceived Stress Scale score	0.97	0.95, 0.99	0.015
IES-R Intrusion subscale score	1.35	1.04, 1.75	0.026
CTX cycle length			0.001
21 day cycle vs 14 day cycle	0.58	0.44, 0.77	< 0.001
28 day cycle vs 14 day cycle	0.91	0.52, 1.61	0.754
21 day cycle vs 28 day cycle	0.64	0.37, 1.11	0.110
Antiemetic regimen			0.019
Steroid alone or serotonin receptor antagonist alone vs None	0.88	0.48, 1.61	0.675
Serotonin receptor antagonist and steroid vs None	1.52	0.87, 2.67	0.141
NK-1 receptor antagonist and two other antiemetics vs None	1.37	0.75, 2.49	0.307
Serotonin receptor antagonist and steroid vs Steroid alone or serotonin receptor antagonist alone	1.73	1.21, 2.49	0.003
Steroid alone or serotonin receptor antagonist alone vs NK-1 receptor antagonist and two other antiemetics	0.64	0.42, 0.97	0.037
Serotonin receptor antagonist and steroid vs NK-1 receptor antagonist and two other antiemetics	1.12	0.80, 1.56	0.529
Overall model fit: df = 13, $X^2 = 189.99$, $p < 0.001$			

Abbreviations: CES-D = Center for Epidemiological Studies-Depression Scale, CI = confidence interval, CTX = chemotherapy, IES-R = Impact of Event Scale-Revised, LFS = Lee Fatigue Scale, NK-1 = neurokinin-1

Chapter 4:

Differentially Expressed Genes and Perturbed Pathways in the Gut-Brain Axis Are Associated With Chemotherapy-Induced Nausea

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ABSTRACT

Purpose: Despite current advances in antiemetic treatments, approximately 50% of oncology patients experience chemotherapy-induced nausea (CIN). The aim of this study, in a sample of oncology patients receiving chemotherapy (CTX), was to evaluate for differentially expressed genes and perturbed pathways associated with the gut-brain axis (GBA) across two independent samples of patients who do and do not experience CIN, after controlling for significant demographic and clinical characteristics.

Experimental Design: Oncology patients (n = 709) completed study questionnaires in the week and prior to their next cycle of 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 test was used to combine the results of the differential gene expression tests and perturbed pathway analyses to determine significant differences between the two CIN groups.

Results: In the combined analyses, 703 differentially expressed (DE) genes (e.g., *C-C motif chemokine receptor 9*, *toll like receptor 5*) and 37 perturbed pathways (e.g., chemokine signaling pathway, intestinal immune network for immunoglobulin A production) were identified. These DE genes and perturbed pathways were related to alterations in mucosal inflammation and disruption of the gut microbiome.

Conclusions: Our study is the first to report on associations between the occurrence of CIN and two mechanisms (i.e., mucosal inflammation and disruption of the gut microbiome) by which CTX can alter the function of the GBA. Additional research is warranted to replicate our findings.

Keywords: chemotherapy; cancer; differential gene expression; pathway perturbation; nausea; mucosal inflammation; gut microbiome

INTRODUCTION

Despite the use of guideline directed antiemetic regimens, nausea continues to be one of the most severe side effects of chemotherapy (CTX).(1) In fact, in our recent study,(2) 48% of patients reported CTX-induced nausea (CIN) prior to their next dose of CTX. While studies have determined a number of phenotypic characteristics associated with unrelieved CIN,(3-6) less is known about the molecular characteristics associated with this symptom.

In a recent review,(7) 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 in 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 (8, 9) and none of the alleles in the cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*) gene (10) were associated with CIN occurrence. Three SNPs and two haplotypes in the ATP binding cassette subfamily B member 1 (*ABCB1*) gene (10-14) 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 established mechanisms for CINV. Therefore, a more exploratory 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).(15-17) Emerging evidence suggests that the administration of CTX results in mucosal inflammation (18-20) and disruption of gut microbiome.(21-23)

In terms of direct effects on the intestinal mucosa, CTX induces the synthesis and release of cytokines that result in mucosal inflammation and disruption of mucosal integrity along the entire gastrointestinal (GI) tract.(16, 19, 24) In addition, CTX-induced mucosal injury alters the

gut microbiome and increases the release of additional inflammatory cytokines.(16, 22, 25, 26) While findings from three clinical studies (15, 17, 27) led the authors to hypothesize that CTX-induced activation of the GBA was associated with CIN, no genomic studies were identified. Therefore, to explore this hypothesis, 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

Patients and settings

This study is part of a longitudinal study, funded by the National Cancer Institute, that evaluated the symptom experience of oncology outpatients receiving CTX.(28, 29) Patients were included if: they were ≥18 years of age; had a diagnosis of breast, GI, gynecological, or lung cancer; had received CTX within the preceding four weeks; were scheduled to receive at least two additional cycles of CTX; were able to read, write, and understand English; and provided written informed consent. Patients were recruited from two Comprehensive Cancer Centers, one Veteran's Affairs hospital, and four community-based oncology programs. A total of 2234 patients were approached and 1343 consented to participate (60.1% response rate). The major reason for refusal was being overwhelmed with their cancer treatment. For this study, 735 patients had gene expression data available.

Study procedures

The study was approved by the Committee on Human Research at the University of California at San Francisco and by the Institutional Review Board at each of the study sites. A research staff member approached eligible patients in the infusion unit and discussed participation in the study. Written informed consent was obtained from all of the patients. Because of the challenges associated with recruitment during the initial cycle of CTX, patients

were recruited during their second or the third cycle of CTX. Depending on the length of their CTX cycle (i.e., 14-day, 21-day, or 28-day), patients completed study questionnaires in their homes, a total of six times over the two cycles of CTX. 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) were used in this analysis to create the nausea groups. Blood for ribonucleic acid (RNA) isolation was collected at the time of the enrollment assessment. Medical records were reviewed for disease and treatment information.

Instruments

Demographic and clinical characteristics - Demographic questionnaire obtained information on: age, gender, ethnicity, marital status, living arrangements, education, employment status, income, and past medical history. Karnofsky Performance Status (KPS) scale was used to evaluate functional status. (30, 31) Self-Administered Comorbidity Questionnaire (SCQ) evaluated the occurrence, treatment, and functional impact of thirteen common comorbid conditions. (32) Total SCQ score ranges from 0 to 39. Alcohol Use Disorders Identification Test (AUDIT) evaluated alcohol consumption, alcohol dependence, and the consequences of alcohol abuse in the last 12 months.(33) Smoking questionnaire assessed smoking history. (34)

Nausea assessment - Memorial Symptom Assessment Scale (MSAS) was used to assess nausea. Patients were asked to indicate whether or not they had experienced nausea in the past week (i.e., symptom occurrence).(35) Patients' assessment of nausea in the week prior to their next cycle of CTX (i.e., enrollment assessment) was used to dichotomize the sample. Patients who provided a rating for occurrence, frequency, severity, and/or distress for the nausea item were coded as having nausea. Patients who indicated "no" to the occurrence item were coded as not having nausea.

Coding of the emetogenicity of the CTX regimens

Using the Multinational Association for Supportive Care in Cancer (MASCC) guidelines,(36-38) each CTX drug in the regimen was classified as having: minimal, low, moderate, or high emetogenic potential. The emetogenicity of the regimen was categorized into one of three groups (i.e., low/minimal, moderate, or high) based on the CTX drug with the highest emetogenic potential. An exception was made if a patient received doxorubicin and cyclophosphamide. When administered separately, doxorubicin and cyclophosphamide are listed as having moderate emetogenic potential.(38) When given together, the combination has high emetogenic potential.

Coding of the antiemetic regimens

Each antiemetic was coded as a neurokinin-1 (NK-1) receptor antagonist, a serotonin receptor antagonist, a dopamine receptor antagonist, anti-psychotic, anti-anxiety, or a steroid. The antiemetic regimens were coded into one of four groups: none (i.e., no antiemetics administered); steroid alone or serotonin receptor antagonist alone; serotonin receptor antagonist and steroid; or NK-1 receptor antagonist and two other antiemetics (e.g., a serotonin receptor antagonist, dopamine receptor antagonist, prochlorperazine, lorazepam, and/or a steroid).

RNA sample preparation

Total RNA was extracted from whole blood collected into PAXgene RNA stabilization tubes and processed using a standard protocol (Qiagen, USA). RNA concentration was measured by NanoDrop UV spectrophotometry (Thermo Fisher Scientific, Waltham, MA). RNA integrity was evaluated using the RNA 6000 Nano Assay (Agilent, USA).(39) Of the 744 patients in this study, 384 had RNA samples processed using RNA-sequencing (RNA-seq; i.e., sample 1) and 360 patients had RNA samples processed using microarray (i.e., sample 2).

RNA-seq library preparation, sequencing, and processing

Total RNA from 384 samples (i.e., n=375 patients, with n=9 replicate samples) were sent to the University of California Davis Genomics Core Facility (Davis, CA, USA) for library preparation and sequencing. Prior to library preparation, 600 nanograms (ng) of total RNA was treated with the Globin-Zero Gold rRNA Removal Kit (Illumina Inc., San Diego, CA) to deplete cytoplasmic ribosomal RNA(40) and human globin mRNA. (41, 42) The globin/ribo depleted RNA was cleaned with Agencourt RNAClean XP (Beckman Coulter, Indianapolis, IN) and the sequencing libraries were prepared with KAPA RNA HyperPrep Kit (Roche Diagnostics Corp., Indianapolis, IN) according to the manufacturer's protocol. Fourteen cycles of polymerase chain reaction (PCR) amplification were used for double six base pair index addition and library fragment enrichment. Prepared libraries were quantified on a Roche LightCycler 480II (Roche Diagnostics Corp., Indianapolis, IN) using KAPA Illumina library quantitative PCR reagents (Roche Diagnostics Corp., Indianapolis, IN).

Sequencing of the 384 samples was done on an Illumina HiSeq 4000 apparatus (Illumina Inc., San Diego, CA). All 384 samples were multiplexed into four pools of 96 samples each, with each sample labeled with a dual-indexed adapter.(43) The sample pools were sequenced on four lanes for 100 cycles of single-end reads with a 1% PhiX v3 control library spike (Illumina Inc., San Diego, CA). Post-sequencing basecall files (bclfiles) were demultiplexed and converted into a FASTQ file format using the bcl2fastq v2.17 software (Illumina Inc., San Diego, CA). Data were posted and retrieved from a secure FTP site hosted by the Core Facility.

RNA-seq data processing was performed based on best practices (44, 45) and our previous experience.(46, 47) Illumina adapters and leading or trailing low quality bases were removed and reads with an average quality per base below 15 in a 4-base sliding window or below a minimum length of 36 bases were removed using Trimmomatic.(48) Individual samples

were inspected with FASTQC (49) and in aggregate with MultiQC.(50) After initial QC, 10 bases were trimmed from the beginning of all reads and reads were re-inspected with FASTQC.

The reference genome was prepared using the GRCh38 assembly (gencode.v24.GRCh38.p5.fa).(51) Transcriptome annotations (n=60,725) were obtained from the Gencode v24 primary assembly (gencode.v24.primary_assembly.annotation.gtf).(51) Trimmed reads were aligned to the annotated reference genome using the STAR aligner.(52) Output alignment files were validated using ValidateSam. Read groups were added to the alignment file using the Picard tool AddOrReplaceReadGroups. Sorted alignment files were inspected using RNA-SeQC(53) and joined for each sample. Abundance of RNA was estimated from the combined aligned reads using featureCounts.(54)

Replicate count data were processed in edgeR.(55) Ensembl transcripts (56) were annotated with Entrez gene ID and symbol.(57) Lowly expressed tags were filtered out by retaining only those tags with $\geq$10/L reads per million (where L is the minimum library size in millions) in at least N samples (where N is the smallest group size). Count estimates were normalized with the trimmed means of M-values (TMM) method.(58) TMM normalization was applied to the dataset in edgeR using calcNormFactors. Data were explored using multi-dimensional scaling (MDS) plots for all samples to identify sample outliers and potential batch effects due to technical artifacts (e.g., RNA integrity number (RIN), date of RNA extraction). The same technician performed all of the RNA extractions in one laboratory. Associations between technical variables and CIN group were assessed using Fisher's Exact Test or a generalized linear model in R. Significance was assessed at a nominal p-value of 0.05.

Microarray hybridization, preprocessing, and normalization

RNA quantified by microarray (n=360 patients in sample 2) was performed as described in our previous studies.(39, 59) Briefly, for each sample, approximately 100 ng of total RNA was

labeled using the Illumina Total Prep RNA Amplification Kit (Thermo Fisher Scientific, Waltham, MA) and hybridized to the HumanHT-12 v4.0 Expression BeadChip (46,538 probes) (Illumina, San Diego, CA). The BeadChips were scanned using the iScan system (Illumina, San Diego, CA) at the University of California, San Francisco Genomics Core Facility. Each HumanHT-12 BeadChip contained 12 sample BeadArrays. Initial quality assessment was performed using BeadArray.(60) Summary level data were calculated from the uncorrected, non-normalized, and non-transformed summary intensities at the probe level with GenomeStudio (Illumina, San Diego, CA). Data preparation and analyses were performed in R (Version 3.3.3) using well-established protocols (61-64) and our previous experience.(39, 59) The quality control procedures were described in detail previously. (59)

Surrogate variable analysis (SVA)

For both the RNA-seq and microarray data, SVA was used to identify technical variations that contributed to heterogeneity in the sample (e.g., batch effects) that were not due to the variable of interest (i.e., nausea group membership) or significant phenotypic covariates.(65) The "be" method was used to identify surrogate variables.(65, 66) Any surrogate variable that was significantly associated with the phenotype was excluded.

Data analyses

Demographic and clinical data – Data were analyzed using SPSS Version 23 (IBM, Armonk, NY). Data from the two patient samples were analyzed separately. Descriptive statistics and frequency distributions were calculated for 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 or Chi-square analysis.

Multiple logistic regression analysis was used to determine significant covariates for inclusion in the DE analysis. Only those characteristics that were significantly different in the

univariate analyses between patients who did and did not report CIN were evaluated in the logistic regression analyses. A backwards stepwise approach was used to create a parsimonious model. Only those characteristics with a p-value of <0.05 were retained in the final model.

Differential GE – For the RNA-seq data, differential GE tests were performed using our previous protocol. (46, 47) Briefly, DE was determined under a variance modeling strategy that addressed the over-dispersion observed in GE count data using edgeR.(67) For this analysis, the overall dispersion, as well as the gene-wise and tag-wise dispersion, were estimated using general linear models estimated using the Cox-Reid (CR)-adjusted likelihood method.(68, 69) Differences in GE between the two CIN groups were tested using likelihood ratio tests. Phenotypic characteristics that differed between the two CIN groups, as well as surrogate variables, were included as covariates in the model.

For the microarray data from sample 2, differential GE tests were performed using our previously published protocol. (39, 70) Briefly, a linear model was fit using the "ls" method which included array weights and significant demographic, clinical, and surrogate variables using limma.(71) The "eBayes" method was used to evaluate for differential expression (DE).(72)

Fisher's Combined Probability test was used to combine the results of the differential GE tests from both datasets.(73, 74) The uncorrected p-values obtained from both datasets (i.e., sample 1 and sample 2) were merged using the ENTREZ gene identifier. Significance of the combined transcriptome-wide GE analysis was assessed using a strict false discovery rate (FDR) of 5% under the Benjamini-Hochberg (BH) procedure.(75) No minimal fold-change was evaluated using the p.adjust R function.

Pathway Impact Analysis (PIA) – Most pathway analyses consider pathways as lists of genes and ignore the additional information available in the pathway representation (e.g., topology).

However, PIA includes potentially important biological factors (e.g., gene-gene interactions, flow signals in a pathway, pathway topologies) as well as the magnitude (i.e., log fold-change) and the p-values from the DE analysis.(76) Using Pathway Express,(77) the PIA included p-values and log fold-changes for all genes that had DE results to determine the probability of a pathway perturbation (pPERT). A total of 208 signaling pathways were defined using the KEGG database.(78) Sequence loci data were annotated with Entrez gene IDs. The gene names were annotated using the HUGO Gene Nomenclature Committee resource database.(79) PIA was performed independently for each dataset (i.e., microarray and RNA-seq).

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.(73, 74) Significance of the combined transcriptome-wide PIA analysis was assessed using a family wise error rate (FWER) of 1% under the Bonferroni method.(77)

RESULTS

Differences in demographic and clinical characteristics

Of the 357 patients in sample 1, 63.6% reported nausea in the week prior to their next cycle of CTX. As shown in Table 4.1, compared to the no nausea group, patients who reported nausea were significantly younger, had a lower KPS score, had a higher comorbidity score, had less time since their cancer diagnosis, had a lower annual income, and were less likely to be employed. Compared to the no nausea group, a lower percentage of patients in the nausea group had two types of cancer treatments and a higher percentage of patients received CTX on a 14-day cycle. 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 patients in sample 2, 48.9% reported nausea in the week prior to their next cycle of CTX. As shown in Table 4.2, compared to the no nausea group, patients who reported nausea had fewer years of education and had 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. A higher percentage of patients in the nausea group 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 no nausea group.

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 GE analyses (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 a 14 day cycle, patients who received a 21 day cycle of CTX 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 GE analyses (Table 4.3). 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.

RNA-seq performance

Of the 375 unique patients in sample 1, 10 samples were determined to be outliers in the MDS plots and 8 did not have phenotypic data. Of the 357 remining patients, 23 patients were excluded due to incomplete demographic and clinical data leaving 334 (n=213 with nausea, n=121 without nausea) for subsequent analyses. Median library size was 9,273,000 reads. Genes with a threshold of $\leq$3.10 (10/L) in all 334 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.(67)

Microarray performance

Of the 360 unique participants in sample 2, four arrays were excluded because of poor hybridization performance across all probes; three arrays were identified as outliers using distance array signal intensity distributions with ArrayQualityMetrics; and one sample did not have phenotypic data. No arrays were excluded because of poor hybridization performance for positive, background negative, and biotin controls assays. Of the 352 patients in the remaining arrays in sample 2, 58 patients were excluded due to incomplete demographic and clinical data leaving 294 arrays (n=140 with nausea, n=154 without nausea) for subsequent analyses.

Background correction, quantile normalization, and log2 transformation were performed using limma. (80) Of the initial probes evaluated for quality (n=46,542), 1953 probes did not have insufficient expression measurements (Illumina detection p-value <0.05) and were excluded, leaving 44,589 probes for analysis.

Differentially expressed genes between the two nausea groups

For sample 1, phenotypic characteristics that differed between the groups (i.e., KPS score, CTX cycle length, and type of prior cancer treatment) were included in the final model for DE. While 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, phenotypic characteristics that differed between the groups (i.e., child care responsibility, KPS score, emetogenicity of CTX, cancer diagnosis) were included in the final model for DE. 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.

Using Fisher's combined probability test, 703 genes were significantly DE at a strict FDR of 5%. Table 4.4 provides a list of differentially expressed genes associated with alterations in the GBA.

Pathway impact analysis

For samples 1 and 2, assays with unique ENTREZ gene identifiers were used in the PIAs (n=20,216 and n=11,577, respectively). Using Fisher's combined probability test, 37 pathways were significantly perturbed using a strict FWER of 1%. Table 4.5 provides a list of perturbed pathways associated with alterations in the GBA.

DISCUSSION

While several lines of preclinical (81) and clinical (15, 17, 27) evidence suggest that CTX-induced activation of the GBA may result in a variety of GI symptoms (e.g., abdominal bloating), our study is the first to present findings that suggest a number of differentially expressed genes and perturbed pathways associated with alterations in the GBA are found in patients with CIN. We organized the discussion of our findings based on two potential mechanisms through which the administration of CTX can induce changes in the function of the GBA that results in CIN namely: mucosal inflammation (18-20) and disruption of the gut microbiome.(22, 23) A growing body of evidence suggests that mucosal inflammation and disruption of the gut microbiome can alter bidirectional communication along the GBA.(82)

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.(81) This epithelial damage results in the release of reactive oxygen species (ROS) that activate nuclear factor-κB (NF-κB).(18) Activation of NF-κB in epithelial and immune cells causes the synthesis and release of inflammatory cytokines.(18) 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-κB signaling and MAPK signaling pathways,(18, 83) as well as continued synthesis and release of inflammatory cytokines, results in the loss of mucosal integrity along the GI tract.(18, 81)

Consistent with the mechanisms cited above, we found perturbations in three pathways that could be involved in mucosal inflammation (i.e., cytokine-cytokine receptor interaction, MAPK signaling, NF-κB signaling). Evidence to support their involvement in GI inflammation

comes from pre-clinical (84-86) and clinical studies.(87) In two preclinical studies, CTX-induced mucositis was associated with an increase in tumor necrosis factor-alpha (TNF-α) immunostaining(84) as well as with increases in the expression of TNF-α and interleukin-6 (IL-6).(86) In terms of NF-κB, in a clinical study of CTX-induced oral mucositis,(87) compared to pre-treatment biopsies, increased oral mucosal staining for NF-κB was found in biopsies following CTX. In terms of the MAPK pathway, in a pre-clinical study of irinotecan-induced intestinal mucositis,(85) this pathway was significantly perturbed as determined by enrichment analysis.

Additional evidence that supports our hypothesis that CIN is associated with GI inflammation comes from our findings regarding differential expression of the *C-C motif chemokine receptor 9* (*CCR9*) gene and perturbation in the chemokine signaling pathway. Chemokines are a family of small proteins that are involved in the recruitment and activation of leukocytes. While they are thought to play a role in acute and chronic inflammation, they are constitutively expressed on mucosal tissues.(88) CCR9, the receptor for the immune cytokine C-C motif chemokine ligand 25 (CCL25) (which is strongly expressed on intestinal glands and crypts), is expressed on $\alpha4\beta7^+$ gut-homing T cell subsets of lamina propria lymphocytes and intraepithelial lymphocytes in the small intestine, as well as on IgA secreting plasma cells found in secondary lymphoid organs.(89) These findings suggest that the CCL25-CCR9 axis may be involved in IgA responses to antigens in the GI tract and resultant mucosal immunity. While not evaluated in the context of CTX-induced mucosal inflammation, compared to healthy controls, in patients with Crohn's disease, CCR9 expression on T cells was increased in peripheral blood but decreased in lamina propria cells of the small intestine.(90)

These alterations in immune function in the gut may affect bidirectional signaling from the gut to the brain and brain to the gut along the GBA.(82) Dysfunction in the bidirectional signaling has been shown to occur in irritable bowel syndrome.(82) Additional research is warranted to evaluate the role of this mechanism in CIN occurrence.

Disruption of the gut microbiome

CTX-induced alterations of the gut microbiome can increase mucosal inflammation through a number of mechanisms, including: influencing the production and release of immunoglobulin A (IgA);(16, 22, 91) constitutive activation of toll-like receptors (TLRs) and related pathways;(92-94) disorganization of tight junctions;(95) and activation of antigen processing and presentation.(96, 97)

In terms of our finding regarding perturbation in the intestinal immune network for IgA production pathway, 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.(16, 91) 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.(98) CTX-induced changes in the gut microflora causes a decrease in the levels of sIgA which results in GI inflammation.(16) Of note, in preclinical studies,(99, 100) treatment with specific bacterial species can increase the synthesis of IgA and decrease GI inflammation. In a recent study of oral mucositis in children receiving CTX for acute leukemia,(101) compared to a control group, mean saliva concentrations of IgA were lower.

A second mechanism by which CTX (e.g., cyclophosphamide (93, 94)) can change the gut microbiome and cause inflammation is through activation of TLRs and related pathways.(92-94) During homeostasis, baseline activation of TLRs occurs through the resident microbiome

present on intestinal epithelial cells (IECs).(102) Preclinical evidence suggests that GI toxins, including CTX causes alterations in TLR signaling.(94, 103) In our study, *TLR5* was differentially expressed and the perturbation value for the TLR signaling pathway was just above our stringent FWER cutoff of $p<0.01$ (i.e., combined pFWER = 0.01175). One can hypothesize that CTX-induced disruption of the microbiome increases levels of flagellin (i.e., a primary structural component of bacterial flagella) which can be recognized by TLR5 and trigger signaling cascades that mediate inflammatory responses.(104) TLR5 mediates signaling through an intracellular adaptor molecule called myeloid differentiation primary-response gene 88 (MyD88) to activate NF-κB signaling pathway.(102, 105, 106) The activation of the NF-κB signaling pathway results in the release of cytokines that increase mucosal inflammation.(102, 105, 106) Of note, we found differential expression of *MyD88* and perturbation in the NF-κB signaling pathway in this study.

TLR5 activation of NF-κB signaling pathway is modulated by *Bacteroids* in the resident microbiome. These bacteria activate the peroxisome-proliferation-activated receptor (PPAR) signaling pathway in the IECs which results in decreased synthesis of pro-inflammatory cytokines and chemokines.(102, 107) Emerging evidence from preclinical studies suggests that this pathway is involved in inflammation, commensal homeostasis, and mucosal immunity in the gut.(108) While we found that the PPAR signaling pathway was perturbed, additional research is needed to confirm its role in CTX-induced alterations in the GBA and CIN.

In addition to IECs, TLR5 is expressed on lamina propria dendritic cells (LPDCs). Activation of these TLRs by SFB is a prerequisite for the differentiation of interleukin 17 (IL-17)-producing T helper (Th17) cells.(109, 110) Recent evidence suggests that the administration of cyclophosphamide favors the growth of segmented filamentous bacteria (SFB) in the gut and enhances the differentiation of Th17 cells and associated increases in serum cytokines.(94, 111)

Given that Th17 cell differentiation is associated with GI inflammation(94, 112) and our finding of a perturbation in the Th17 cell differentiation pathway, its association with CTX-induced alterations in the GBA warrant additional investigation.

Consistent with our finding of a perturbation in the tight junction pathway, a third mechanism by which CTX-induced alterations in the gut microbiome may alter the function of the GBA is by influencing the synthesis of tight junction proteins.(95) CTX can increase intestinal permeability in two ways.(95) First, CTX-induced release of TNF-α downregulates synthesis of tight junction proteins to increase epithelial permeability.(113) Second, CTX can decrease the number of bacteria that regulates the synthesis of tight junction proteins that results in increased epithelial permeability.(16, 95) Evidence from a number of clinical studies suggests that, 5-FU, doxorubicin, and mitomycin (FAM);(114) oxaliplatin, folinic acid, and 5-FU (FOLFOX);(114) or methotrexate(115) disrupt tight junctions and increase intestinal permeability. Of note, in two systematic reviews, the authors concluded that evidence supports the use of glutamine (an amino acid that decreases intestinal permeability) to prevent treatment-related mucositis in patients with cancer(116) and to decrease complications (e.g., mucositis, diarrhea) associated with colorectal cancer treatment.(117)

The fourth mechanism by which CTX-induced changes in the gut microbiome can result in alterations in the GBA is related to our finding of a perturbation in the antigen processing and presentation pathway. The antitumor activity of CTX increases levels of tumor-derived peptide antigens (TDPAs).(118) Translocation of TDPAs and the gut microbiome into the permeable intestine activates antigen presenting dendritic cells (APDCs) in the lamina propria.(96) APDCs adjust the adaptive immune response based on changes in the intestinal environment.(94, 96) In addition, IECs function as antigen presenting cells and activate T cells in the lamina propria that are involved in downstream inflammatory processes.(97, 119) Of note and related to our finding

of differential expression of *heat shock family protein D (Hsp60) membrane 1* (*HSPD1*), extracellular HSPD1 interacts with TLRs to present TDPAs to immune cells and induces the release of cytokines.(120-122) Activation of the antigen processing and presentation pathway in IECs and APDCs results in the release of inflammatory cytokines which aggravates GI inflammation.(96, 97, 119) While Hsp60 is being investigated as a novel target to treat cancer (122-124), 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 GE and pathway perturbation selection, and the combination of results from independent tests across two samples, several limitations warrant consideration. While we have indirect evidence from blood samples to support our hypothesis that CTX-induces changes in the GBA, future studies are warranted that obtain tissue samples along the GI tract to provide direct evidence for associations between CIN and alterations in mucosal inflammation and disruption in the gut microbiome. While our sample was large and representative of patients with CIN, our findings warrant confirmation in an independent cohort. Given that our phenotype and GE measures were done prior to the next cycle of CTX, additional research is warranted to determine if these changes in GE and pathway perturbations occur at other time points during the administration of CTX.

Conclusions and directions for future research

Despite these limitations, our study is the first to report on associations between the occurrence of CIN and two mechanisms (i.e., mucosal inflammation and disruption of gut microbiome) by which CTX can alter the function of the GBA. Findings from several clinical studies support an association between CTX-induced changes in the GBA and a number of GI symptoms.(15, 17, 27) As shown in Table 4.6, we evaluated for differences between patients

with and without CIN, in the occurrence of eleven GI symptoms listed on the MSAS. Patients with CIN reported higher occurrence rates for all of the GI symptoms evaluated (e.g., change in the way food tastes, lack of appetite, dry mouth). Our findings suggest that additional research is warranted to evaluate the complex mechanisms that underlie the occurrence of CIN. In addition, future research needs to determine the relationships among other GI symptoms and their associated mechanisms and the occurrence and severity of CIN.

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Table 4.1 – 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^2)	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			FE, p = 0.290
Female	74.6 (97)	79.7 (181)	
Male	25.4 (33)	20.3 (46)	
Ethnicity			X^2 = 3.62, p = 0.305
White	68.2 (88)	60.4 (137)	
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
Currently employed (% yes)	41.4 (53)	30.5 (69)	FE, p = 0.048
Income			U, p = 0.041
< $30,000	12.5 (14)	27.1 (57)	
$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			X^2 = 4.46, p = 0.216
Breast	41.5 (54)	38.3 (87)	
Gastrointestinal	31.5 (41)	37.0 (84)	
Gynecological	20.0 (26)	13.7 (31)	
Lung	6.9 (9)	11.0 (25)	

Characteristic	No Nausea 36.4% (n = 130)	Nausea 63.6% (n = 227)	Statistics
	% (n)	% (n)	
Type of prior cancer treatment			$X^2 = 11.28$, 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			$X^2 = 8.23$, 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			$X^2 = 2.17$, p = 0.337
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^2 = 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^2 = Chi square

Table 4.2 – 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^2)	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			FE, p = 0.590
Female	79.4 (143)	82.0 (141)	
Male	20.6 (37)	18.0 (31)	
Ethnicity			X^2 = 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
Currently employed (% yes)	33.0 (59)	35.7 (61)	FE, p = 0.653
Income			U, p = 0.001
< $30,000	15.5 (25)	27.6 (43)	
$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^2 = 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

Characteristic	No Nausea 51.1% (n = 180) % (n)	Nausea 48.9% (n = 172) % (n)	Statistics
Type of prior cancer treatment			$X^2 = 1.38$, 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			$X^2 = 10.30$, 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			$X^2 = 8.05$, p = 0.018
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			$X^2 = 15.65$, 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^2 = 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^2 = Chi square

Table 4.3 – Multiple Logistic Regression Analysis 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 4.4 – Differentially Expressed Gut-Brain Axis Related Genes Between Oncology Patients With and Without Chemotherapy-Induced Nausea

Ensemble Gene ID	Microarray Probe ID	Entrez ID	Gene Symbol	Name	pGlobal.FDR
Mucosal Inflammation					
ENSG00000173585	ILMN_1664316	10803	*CCR9*	chemokine receptor 9	0.012
Disruption of gut microbiome					
ENSG00000187554	ILMN_1722981	7100	*TLR5*	toll-like receptor 5	0.012
ENSG00000172936	ILMN_1738523	4615	*MyD88*	myeloid differentiation primary response 88	0.038
ENSG00000144381	ILMN_1797398	3329	*HSPD1*	heat shock family protein D (Hsp60) membrane 1	0.023

Abbreviation: FDR = false discovery rate

Table 4.5 – 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 κB signaling pathway*	0.00982
hsa04062	Chemokine signaling pathway	0.00084
Disruption of gut microbiome		
hsa04672	Intestinal immune network for immunoglobulin A production	0.00917
hsa04620	Toll like receptor signaling pathway	0.01175
hsa04064	Nuclear factor κB 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

Table 4.6 – Differences in the Occurrence of Gastrointestinal Symptoms Between Patients With and Without Chemotherapy-Induced Nausea

Gastrointestinal Symptom (% yes)	No Nausea 52.6% (n = 698)	Nausea 47.4% (n = 629)	Statistics
	% (n)	% (n)	
Change in the way food tastes	38.3(267)	61.5(387)	FE, $p < 0.001$
Lack of appetite	24.4 (170)	60.1 (378)	FE, $p < 0.001$
Dry mouth	33.5 (234)	58.5 (368)	FE, $p < 0.001$
Constipation	32.4 (226)	55.6 (350)	FE, $p < 0.001$
Feeling bloated	25.1 (175)	42.0 (264)	FE, $p < 0.001$
Diarrhea	21.6 (151)	38.2 (240)	FE, $p < 0.001$
Weight loss	16.8 (117)	34.7 (218)	FE, $p < 0.001$
Abdominal cramps	13.8 (96)	32.1 (202)	FE, $p < 0.001$
Mouth sores	15.0 (105)	27.5 (173)	FE, $p < 0.001$
Vomiting	1.6 (11)	24.3 (153)	FE, $p < 0.001$
Difficulty swallowing	7.3(51)	20.7(130)	FE, $p < 0.001$

Abbreviation: FE = Fisher's Exact test

Chapter 5:

Conclusions for Dissertation

The purposes of this dissertation research were to: perform a systematic review of the literature on the associations between single nucleotide polymorphisms (SNPs) in candidate genes and the occurrence of CIN; determine additional risk factors associated with the occurrence of CIN; and determine additional molecular mechanisms associated with occurrence of CIN.

Chapter one provides a review of the current predictors for CIN; a description of the types of CIN and the mechanisms that underlie the development of CIN; and a brief summary of current approaches to antiemetic prophylaxis for CIN. Despite our current knowledge of predictors, mechanisms, and treatments, CIN continues to be a significant clinical problem. Between 30% and 60% of oncology patients experience CIN. In a multinational study that investigated the incidence of acute and delayed CINV in patients receiving moderately and highly emetogenic CTX treatment regimens,(1) over 35% of the patients experienced acute nausea. In addition, 52% of the patients who received moderately emetogenic CTX and 60% of patients who received highly emetogenic CTX experienced delayed nausea. These studies suggest that our current state of knowledge of the mechanisms that underlie the occurrence of CIN warrants additional investigation. This dissertation research focuses on investigating risk factors associated with the occurrence of CIN and mechanisms related to the GBA axis that may be involved in the occurrence of CIN.

In Chapter two, sixteen studies were reviewed that investigated associations between various CINV phenotypes and polymorphisms in a number of candidate genes.(2) Across these studies, three SNPs in 5-hydroxytryptamine receptor (*5-HT3R*) genes, two alleles of the cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*) gene, and three SNPs in the ATP

binding cassette subfamily B member 1 (*ABCB1*) gene were associated with the occurrence and severity of CINV.

In Chapter three, demographic, clinical, symptom, and stress characteristics associated with an increased risk for the occurrence of CIN are presented.(3) These risk factors include: less education; having child care responsibilities; poorer functional status; higher levels of depression, sleep disturbance, evening fatigue, and intrusive thoughts; as well as receipt of CTX on a 14-day CTX cycle and receipt of an antiemetic regimen that contained a serotonin receptor antagonist and a steroid. Patients in the CIN group experienced clinically meaningful decrements in QOL.

In Chapter four, evidence is provided for associations between a number of differentially expressed genes and perturbed pathways in the GBA and the occurrence of CIN. CTX-induced changes in the GBA that may contribute to the occurrence of CIN include: mucosal inflammation and disruption of gut microbiome.

Implications for Clinical Practice

In our descriptive study,(3) 48% of oncology patients reported nausea in the week prior to their next cycle of CTX. Patients who reported CIN had not only statistically significant but clinically meaningful decrements in overall QOL. The modifiable risk factors that were associated with CIN group membership included: having child care responsibilities; poorer functional status; and higher levels of depression, sleep disturbance, evening fatigue, perceived stress, and intrusive thoughts and feelings. Clinicians need to assess patients for these risk factors and refer them for appropriate interventions (e.g., physical therapy, mental health services). Clinicians need to educate patients about stress reduction strategies and the importance of adhering with the antiemetic regimen.

While current anti-emetic regimens are based on our understanding of the mechanisms associated with CINV (i.e. serotonin receptor pathway, drug transport pathway and drug metabolism pathway), our data (3) and work of others(4, 5) suggest that CIN continues to be a significant clinical problem. Of note, findings from our systematic review found that associations between candidate genes selected based on these established mechanisms and occurrence of CIN remain inconclusive.(2) Therefore, a hypothesis-generating study was undertaken to uncover novel mechanisms associated with the occurrence of CIN. Our findings suggest that CIN-induced changes in the GBA occur through mucosal inflammation and disruption of the microbiome. While these findings warrant replication, they provide direction for future clinical trials to decrease the occurrence of CIN (e.g., use of steroids, use of probiotics).

Recommendations for Future Research

Given that our study is the first to evaluate for associations between a comprehensive set of demographic and clinical characteristics, as well as symptom severity scores, and levels of perceived stress and the occurrence of nausea in the week prior to the patient's next cycle of CTX,(3) future studies are warranted to confirm our findings, as well as findings from other clinical studies.(6, 7) Of particular interest given the findings from Chapter 4, additional risk factors for CIN that warrant investigation include an evaluation of the inflammatory state of the GI tract and the profile of the microbiome prior to initiation of CTX.

Moreover, future studies using instruments specifically designed to measure CIN occurrence and severity (e.g. MASCC Antiemesis Tool,(8) Morrow Assessment of Nausea and Emesis Follow-Up (9)) are needed to refine the CIN phenotype. The use of these measures would provide a comprehensive evaluation of anticipatory, acute, and delayed nausea, as well as the effectiveness of the antiemetic regimen.

Patient adherence with the antiemetic regimen needs to be evaluated to determine its association with CIN occurrence, severity, and distress. Longitudinal studies are warranted to identify phenotypic and molecular characteristics that are associated with inter-individual variability in the occurrence and severity of CIN. Because severe nausea can have a negative impact on patients' nutritional status and physical functioning,(10) future studies need to examine these relationships over multiple cycles of CTX. This knowledge will assist clinicians to recommend more targeted interventions to decrease the occurrence and severity of CIN.

Future research is warranted to investigate genetic polymorphisms that are guided by the findings from our GE analysis. In addition, an epigenetic study that is guided by our findings from the GE analysis may provide information about changes in levels of functional gene products in relationship to environmental influences. Given that our phenotype and GE measures were done prior to the next cycle of CTX, additional research is warranted to determine if these changes in GE and pathway perturbations occur at other time points during the administration of CTX.

Additional research is warranted to evaluate the complex mechanisms that underlie the occurrence of CIN. Patients in the CIN group experienced the occurrence of a number of GI symptoms (e.g, change in the way food tastes, lack of appetite, dry mouth). An important area of future research includes investigations of the mechanisms associated with occurrence and severity of CIN as well as the occurrence and severity of other GI symptoms.

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