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Original Article

The Association between ATP-Binding Cassette C2 (ABCC2) Transporter Genetic Polymorphism and Peripheral Neuropathy in Gastrointestinal Cancer Patients Receiving Oxaliplatin

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ABSTRACT

Single nucleotide polymorphisms (SNPs) in the ATP-binding cassette C2 (ABCC2) gene increased intracellular oxaliplatin accumulation in the dorsal root ganglia may result in an increased risk of oxaliplatin-induced peripheral neuropathy (OXAIPN). The present study aims to study the association between the SNPs rs1885301 G>A, rs4148396 C>T, and rs3740066 C>T in the ABCC2 gene and the incidence of OXAIPN in gastrointestinal cancer patients. The study was a prospective cohort study carried out at the Clinical Oncology Department, Ain Shams University Hospitals. Eligible patients received FOLFOX6 and FOLFIRINOX for 8-12 cycles. The SNPs assessment was performed using Real-time PCR using the Rotor gene Q (QIAGEN®) system. The patients were followed up with each cycle to assess the incidence and severity of OXAIPN and other common toxicities including diarrhea, vomiting, and neutropenia. One hundred and twenty patients were included in the study. The minor allele frequency for the SNPs rs1885301 G>A, rs4148396 C>T, and rs3740066 C>T were 0.4-0.2, and 0.3 respectively. The current study showed no association between the three SNPs and the incidence and grade of OXAIPN with less than 50% of the participants reporting grade III and IV peripheral neuropathy. A significant association was found between rs4148396 C>T and the occurrence of neutropenia where TT haplotype showed a significantly higher incidence of neutropenia compared to CC + CT. In conclusion, no association was found between the SNPs and the occurrence of PN, diarrhea, and vomiting. There was only a significant difference in the incidence grades of neutropenia among haplotypes of rs4148396 C>T.

Keywords: *ABCC2 Transporter; Genetic Polymorphism; Peripheral neuropathy; Gastrointestinal Cancer; Oxaliplatin.*

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1. Introduction

Approximately 20% of all cancer diagnoses and 22.5% of all cancer-related deaths globally are attributable to gastrointestinal malignancies **[1]**. Oxaliplatin, particularly in conjunction with 5-fluorouracil (5-FU), is widely used to treat colorectal, pancreatic, gastric, and esophageal cancer **[2-4]**.

Oxaliplatin is linked to many toxicities, peripheral neuropathy (PN) being the most prevalent **[5]**. Oxaliplatin-induced peripheral neuropathy (OXAIPN) can be characterized as either acute or chronic PN **[6]**. Acute OXAIPN occurs in 85-95% of patients and is characterized by cold-sensitive peripheral paresthesia with

tingling, numbness, pressure, or cold or warm anomalous skin sensations, which can begin a few hours after oxaliplatin administration and last for several days **[7]**. Chronic OXAIPN affects 10-15% of oxaliplatin-treated patients and often develops after a cumulative dose of 540-850 mg/m^2. It is characterized by chronic neuropathic symptoms that are not improved by treatment, sensory loss, and proprioceptive changes that may interfere with everyday activities. After discontinuing oxaliplatin, the signs and symptoms of chronic OXAIPN may subside during the next 6-12 months, or they may continue for several years **[8, 9]**. Oxaliplatin buildup in the dorsal root ganglia (DRG) is associated with the pathogenesis of OXAIPN. Oxaliplatin can generate DNA adducts with the DNA of neuron cell organelles such as the nucleus and mitochondria. These changes can disrupt DNA replication and the cell cycle, hamper DNA repair, and cause neuronal death **[10]**.

Numerous genes regulate the pharmacokinetic and pharmacodynamic aspects of oxaliplatin, and many single nucleotide polymorphisms (SNPs) in these genes have been linked to OXAIPN **[11]**. Several solute transporters, notably ATP-binding cassette (ABC) transporters, have been connected to the influx or efflux of chemotherapeutic medicines containing platinum, such as oxaliplatin **[12]**. ABC-transporter superfamily may give innate or acquired multidrug resistance (MDR) by expelling anticancer medicines or their metabolites from cells, while blockage of such transporters may result in cytostatic agent sensitization **[13]**. The MDR protein 2 (MRP2), encoded by the (ABCC2) gene, functions as an ATP-dependent efflux pump in the apical membrane of many polarized cells **[14]**.

Certain SNPs in the ABCC2 gene have been associated with increased intracellular oxaliplatin accumulation in the DRG, hence raising the risk of OXAIPN **[15]**. Prior research conducted on the European population evaluated the influence of genetic polymorphisms of ABCC2 including rs3740066 C>T, rs1885301 G>A, and rs4148396 C>T, and reported a link between the rare alleles and the elevated risk of OXAIPN **[5]**. No pharmacogenetic investigations are available to discover the influence of these SNPs on the occurrence of OXAIPN in the Egyptian population. Consequently, the goal of the present study was to explore the impact of the SNPs rs1885301 G>A, rs4148396 C>T, and rs3740066 C>T of the ABCC2 gene on the incidence and severity of OXAIPN in Egyptian gastrointestinal cancer patients.

2. Patients and Methods

2.1. Methodology

This prospective cohort study was undertaken by the Clinical Oncology Department of Ain Shams University Hospitals on 120 Egyptian patients with gastrointestinal cancer who received oxaliplatin-based chemotherapy. The study was done following the 1964 Declaration of Helsinki and its 2013 revision. The research protocol with approval number was amended and authorized by the research ethics committee for experimental and clinical studies at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt: Master (Eneric – ASU .2020-297). A participant's informed permission was obtained before inclusion in the study.

2.2. Patients

The eligibility of all cancer patients presenting to the department was evaluated. Chemotherapy-naive adult patients, diagnosed with gastrointestinal cancer, and scheduled to receive oxaliplatin-based chemotherapy {FOLFOX6 & FOLFIRINOX as oxaliplatin dose is 85 mg/m^2}met the inclusion criteria **[16, 17]**, sufficient bone marrow function {absolute

neutophil count (ANC)>1500 cells/mm3}, renal function {estimated glomerular filtration rate (eGFR)>60 mL/min}, liver function {alanine transaminase test (ALT) and aspartate aminotransferase (AST) up to three times upper normal limits} and an Eastern Cooperative Oncology Group performance status of ≤2. Patients with clinical neuropathy, diabetic mellitus, or other primary malignancies, as well as those who were pregnant or breastfeeding, were excluded from the study.

2.3. SNP selection and DNA Extraction

Due to a lack of information on the allelic frequencies of distinct genes in the Egyptian population, it was postulated in this study that its distribution is comparable to that of the European and African populations, given Egypt's geographic location concerning Africa and Europe. Minor allele frequency was utilized to select SNPs in the ABCC2 gene: rs1885301 G>A (MAF=0.38) **[18]**, rs4148396 C>T (MAF=0.27) **[19],** and rs3740066 C>T (MAF=0.28) **[20]**.

2.4. Methods

Patients who qualified for the trial were informed of the study protocol. Before initiating oxaliplatin-based chemotherapy, each patient's blood was collected in vacutainer tubes containing Ethylenediaminetetraacetic acid (EDTA) and stored at -80 °C as whole blood until extraction. Each cycle, patients were evaluated clinically for the presence of OXAIPN. In addition, they were instructed on the signs and symptoms of OXAIPN and asked to report their occurrence. Each cycle, patients were evaluated for the occurrence of additional toxicities, including diarrhea, vomiting, and neutropenia. Toxicities were rated using the National Cancer Institute's Common Toxicity Criteria for Adverse Event (NCI- CTCAE V4.0) (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_40).

2.5. Deoxyribonucleic acid isolation and SNP assessment

DNA is isolated using the QIAamp DNA Blood Mini Kit (QIAGEN®, Hilden, Germany) per the manufacturer's guidelines. TaqMan® SNP (Applied Biosystems®, genotyping test, Waltham, Massachusetts, USA) and TaqMan Universal PCR Master Mix (Applied Biosystems®, Waltham, Massachusetts, USA) were utilized to detect genetic polymorphism (rs3740066, rs1885301, and rs4148396). Real-time PCR was done using Rotor gene Q (QIAGEN®, Hilden, Germany).

2.6. Primary endpoint

The study's primary purpose was to determine the relationship between rs3740066 C>T, rs1885301 G > A, and rs4148396 C>T with the occurrence and severity of OXAIPN in patients with gastrointestinal cancer who received oxaliplatin.

2.7. Secondary endpoint

The evaluation of the connection of these SNPs with the incidence of other toxicities.

2.8. Statistical analysis

Version 4.2.2 of the R statistical program was utilized to conduct statistical analysis. Shapiro test of normality was performed to test the normality of the continuous variables described by their median and interquartile range (IQR). Furthermore, the categorical variables are described by their counts and percentages. The chi-square test was used to determine if the genotype frequency distributions matched those predicted by Hardy-Weinberg equilibrium. For comparing categorical data, the Chi-square test was utilized. The Kruskal-Wallis rank sum test was used for the comparison of numerical data. Kaplan-Meier analysis was used to compare the time required to develop OXAIPN and the p-value was generated using the log-rank test. P-

values less than 0.05 were considered significant.

2.9. Sample size calculation

Using the PASS 15 tool for sample size calculation, analyzing results from a previous study. A previous study found a 6.9% incidence of grade III and IV PN in colorectal cancer patients treated with the FOLFOX-4 regimen. The study would require a sample size of 120 to obtain a two-sided 95% confidence interval with a width of 0.100 when the sample proportion is 0.069 **[5]**.

3. Results

3.1. Baseline characteristics

From August 2021 to October 2022, 140 patients were evaluated for eligibility and 120 patients met the eligibility requirements and were recruited in the study. The overall median age (IQR) of patients in the study group was 53 years (IQR: 43 to 60 years). 53% of the subjects were female, with a median (IQR) BSA of 1.75 inches (1.65-1.91). At baseline, all patients had normal liver and kidney function. Hypertension (43.3%) and asthma (32.5%) were the most often reported co-morbidities. The majority of patients (55 %) had colon cancer, and 65 % of them were in the metastatic stage. Approximately 76% of the subjects received the FOLFOX-6 regimen. rs3740066 C>T, rs1885301 G>A, and rs4148396 C>T each had minor allele frequencies of 0.3-0.4, and 0.2 respectively. The participant's characteristics at baseline are shown in **Table 1**.

The ABCC2 rs4148397 C>T, rs3740066 C>T, and rs1885301 G>A all match those predicted by Hardy-Weinberg equilibrium (p=0.015, 0.373, and 0.997, respectively).

Except for the chemotherapy procedure in rs1885301 G>A, there were no significant demographic and baseline clinical characteristics differences among the three haplotypes in rs4148396, rs3740066, and rs1885301. **Tables 2** and **Tables 3** summarize these statistics.

3.2. Assessment of OXAIPN

The incidence of the various grades of OXAIPN was as follows: grade I (16.7%), grade II (49.2%), grade III (26.7%), and grade IV (7.5%). There was no significant difference in the incidence and grading of OXAIPN difference among three haplotypes in rs4148396 C>T, rs3740066 C>T, and rs1885301 G>A as reported in **Table 4**.

The Kaplan-Meier curve for the development of grade III and grade IV OXAIPN is shown in **Fig.1**. Using the log-rank test, there was no significant difference across haplotypes for each SNP (p-values of 0.500, 0.400, and 0.600, respectively, for rs4148396 C>T, rs3740066 C>T, and rs1885301 G>A).

3.3. Incidence and grading of other toxicities

The frequency of neutropenia, vomiting, and diarrhea was documented in **Table 5**. The incidence of neutropenia was as follows: grade I (11.7%), grade II (61.7%), grade III (25.8%), and grade IV (0.8%). There was only a statistically significant distinction in the occurrence of different stages of neutropenia among haplotypes of rs4148396 C>T, where TT had a 45.5% higher prevalence of grade III and IV neutropenia than CC+CT (24.8%).

According to the current study, the incidence of vomiting in grades III and IV was 16.7% and 4.2%, while the incidence of diarrhea in grades III and IV was 20% and 6.7%, respectively. Among the three haplotypes no significant differences in rs4148396 C>T, rs3740066 C>T, and rs1885301 G>A in terms of vomiting and diarrhea severity.

Table 1. Descriptive statistics for the study participants (n=120)

Characteristics	
Gender, n (%)	
Male	56 (46.7%)
Female	64 (53.3%)
Age in years, median (IQR)	53 (43- 60)
Body Surface Area (BSA) in m2, median (IQR)	1.76 (1.66-1.91)
ALT (U/L), median (IQR)	27 (22-33)
AST (U/L), median (IQR)	25 (20-32)
Serum Creatinine (mg/dl), median (IQR)	1.04 (1.00 - 1.09)
Hypertension: n (%)	52 (43.3%)
Asthma: n (%)	39 (32.5%)
Type of cancer: n (%)	
Colon	66 (55.0%)
Rectal	22 (18.3%)
Gastric	12 (10.0%)
Pancreatic	17 (14.2%)
Esophageal	3 (2.5%)
Stage of Cancer: n (%)	
II	7 (5.8%)
III	35 (29.2%)
IV	78 (65.0%)
Chemotherapy Protocol: n (%)	
FOLFOX	91 (75.8%)
FOLFIRINOX	29 (24.2%)
Goal of Protocol: n (%)	
Adjuvant	42 (35%)
Metastatic	78 (65%)
Number of cycles: n (%)	
9	5 (4.2%)
10	22 (18.3%)
11	1 (0.8%)
12	92 (76.7%)
rs4148396: n (%)	
CC	72 (60%)
CT	37 (30.8%)
TT	11 (9.2%)
rs3740066: n (%)	
CC	66 (55%)
CT	43 (35.8%)
TT	11 (9.2%)
rs1885301: n (%)	
AA	19 (15.83%)
AG	58 (48.33%)
GG	43 (35.83%)

BSA, body surface area; IQR, interquartile range; ALT, alanine transaminase test; AST, aspartate aminotransferase test.

Table 2. Baseline demographic and clinical characteristics distributed among the three haplotypes in each SNP

Characteristic	rs4148396			*P*	rs3740066			*P*	rs1885301			*P*
	CC n=72	CT n=37	TT n=11		CC n=66	CT n=43	TT n=11		AA n=19	AG n=58	GG n=43	
Gender				0.491[a]				0.286[a]				٠.٦٠٨[a]
Male: n (%)	32 (44.4%)	20 (54.1%)	4 (36.4%)		30 (45.5%)	23 (53.5%)	3 (27.3%)		7 (36.8%)	29 (50.0%)	20 (46.5%)	
Female: n (%)	40 (55.6%)	17 (45.9%)	7(63.6%)		36 (54.5%)	20 (46.5%)	8 (72.7%)		12 (63.2%)	29 (50.0%)	23 (53.5%)	
Age in years,				0.459[b]				0.147[b]				0.546[b]
Median	54	53	45		53	54	44		50	54	53	
IQR	(44 - 60)	(43 - 60)	(43 - 52)		(43-60)	(48- 61)	(40- 50)		(44- 54)	(44 - 61)	(42 - 58)	
BSA in m2,				0.173[b]				0.909[b]				0.129[b]
Median	1.73	1.73	1.8		1.74	1.76	1.8		1.8	1.76	1.71	
(IQR)	(1.63 -1.90)	(1.70 - 1.90)	(1.79- 1.99)		(1.62- 1.94)	(1.70-1.90)	(1.68-1.90)		(1.70- 1.99)	(1.68 - 1.90)	(1.59-1.87)	
ALT (U/L),				0.055[b]				0.230[b]				0.284[b]
Median	28	25	23		27	25	28		23	26	28	
IQR	(23 - 34)	(21 - 31)	(21- 28)		(23- 34)	(21-30)	(24-32)		(21 - 28)	(21- 34)	(24 - 33)	
AST (U/L),				0.481[b]				0.544[b]				0.938[b]
Median	25	26	21		24	26	25		25	25	23	
IQR	(20 - 31)	(21 - 32)	(19- 30)		(20-29)	(21-33)	(19-31)		(20- 33)	(20- 30)	(21- 30)	
S.Cr (mg/dl),		1.04	1	0.275[b]				0.151[b]		1.04		0.176[b]
Median	1.04	(1.00 -	(1.00-		1.04	1.04	1		1	(1.00-	1.05	
IQR	(1.00 -1.09)	1.10)	1.03)		(1.00-1.09)	(1.00-1.10)	(0.95- 1.02)		(0.95- 1.03)	1.09)	(1.00- 1.10)	
Hypertension:				0.211[c]				0.223[c]				0.243[c]
n (%)	34 (47.2%)	16 (43.2%)	2 (18.2%)		31 (47.0%)	19 (44.2%)	2 (18.2%)		5 (26.3%)	28 (48.3%)	19 (44.2%)	
Asthma:				0.835[c]				0.153[c]				0.467[c]
n (%)	25 (34.7%)	11 (29.7%)	3 (27.3%)		25 (37.9%)	13 (30.2%)	1 (9.1%)		5 (26.3%)	22 (37.9%)	12 (27.9%)	

IQR, interquartile range; BSA, body surface area; ALT, alanine transaminase test; AST, aspartate aminotransferase test; S. Cr, serum creatinine; [a]Pearson's Chi-squared test; [b]Kruskal-Wallis rank sum test; [c]Fisher's exact test; p-value<0.05 is significant.

Table 3. Cancer characteristics distributed between the three haplotypes in each SNP

Characteristic	rs4148396			P	rs3740066			P	rs1885301			P
	CC n=72	CT n=37	TT n=11		CC n=66	CT n=43	TT n=11		AA n=19	AG n=58	GG n=43	
Type of cancer				0.862[a]				0.705[a]				0.517[a]
Colon: n (%)	42 (58.3%)	19 (51.4%)	5 (45.5%)		38 (57.6%)	21 (48.8%)	7 (63.6%)		11 (57.9%)	33 (56.9%)	22 (51.2%)	
Rectal: n (%)	13 (18.1%)	6 (16.2%)	3 (27.3%)		13 (19.7%)	8 (18.6%)	1 (9.1%)		4 (21.1%)	9 (15.5%)	9 (20.9%)	
Gastric: n (%)	5 (6.9%)	5 (13.5%)	2 (18.2%)		4 (6.1%)	7 (16.3%)	1 (9.1%)		1 (5.3%)	9 (15.5%)	2 (4.7%)	
Pancreatic: n (%)	10 (13.9%)	6 (16.2%)	1 (9.1%)		10 (15.2%)	5 (11.6%)	2 (18.2%)		3 (15.8%)	5 (8.6%)	9 (20.9%)	
Esophageal: n (%)	2 (2.8%)	1 (2.7%)	0 (0.0%)		1 (1.5%)	2 (4.7%)	0 (0.0%)		0 (0.0%)	2 (3.4%)	1 (2.3%)	
Stage of Cancer				0.894[b]				0.307[b]				0.408[b]
II: n (%)	5 (6.9%)	2 (5.4%)	0 (0.0%)		5 (7.6%)	1 (2.3%)	1 (9.1%)		2 (10.5%)	2 (3.4%)	3 (7.0%)	
III: n (%)	21 (29.2%)	12 (32.4%)	2 (18.2%)		22 (33.3%)	12 (27.9%)	1 (9.1%)		4 (21.1%)	21 (36.2%)	10 (23.3%)	
IV: n (%)	46 (63.9%)	23 (62.2%)	9 (81.8%)		39 (59.1%)	30 (69.8%)	9 (81.8%)		13 (68.4%)	35 (60.3%)	30 (69.8%)	
Chemotherapy Protocol				0.586[b]				0.595[b]				0.028[*b]
FOLFOX-6: n (%)	56 (77.8%)	28 (75.7%)	7 (63.6%)		50 (75.8%)	34 (79.1%)	7 (63.6%)		13 (68.4%)	50 (86.2%)	28 (65.1%)	
FOLFIRINOX: n (%)	16 (22.2%)	9 (24.3%)	4 (36.4%)		16 (24.2%)	9 (20.9%)	4 (36.4%)		6 (31.6%)	8 (13.8%)	15 (34.9%)	
Goal of Protocol				0.465[a]				0.577[a]				0.452[a]
Adjuvant: n (%)	26 (36.1%)	14 (37.8%)	2 (18.2%)		27 (40.9%)	13 (30.2%)	2 (18.2%)		6 (31.6%)	23 (39.7%)	13 (30.2%)	
Metastatic: n (%)	46 (63.9%)	23 (62.2%)	9 (81.8%)		39 (59.1%)	30 (69.8%)	9 (81.1%)		13 (68.4%)	35 (60.3%)	30 (69.8%)	
Number of cycles				0.371[a]				0.517[a]				
9: n (%)	2 (2.8%)	2 (5.4%)	1 (9.1%)		3 (4.5%)	1 (2.3%)	1 (9.1%)		2 (10.5%)	1 (1.7%)	2 (4.7%)	0.259[a]
10: n (%)	10 (13.9%)	10 (27.0%)	2 (18.2%)		9 (13.6%)	11 (25.6%)	2 (18.2%)		3 (15.8%)	14 (24.1%)	5 (11.6%)	
11: n (%)	1 (1.4%)	0 (0.0%)	0 (0.0%)		1 (1.5%)	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	1 (2.3%)	
12: n (%)	59 (81.9 %)	25 (67.6 %)	8 (72.7%)		5 (80.3%)	31 (72.1%)	8 (72.7%)		14 (73.7%)	43 (74.1%)	35 (81.4%)	

[a]Pearson's Chi-squared test; [b]Fisher's exact test; p-value < 0.05 is significant; * Indicates significance.

Table 4. Incidence and grading of peripheral neuropathy distributed among the three haplotypes in each SNP

Toxicity	rs4148396 CC n=72	CT n=37	TT n=11	*P* [a]	rs3740066 CC n=66	CT n=43	TT n=11	*P* [a]	rs1885301 AA n=19	AG n=58	GG n=43	*P* [a]
PN				0.962				0.408				0.858
I: n (%)	12 (16.7%)	6 (16.2%)	2 (18.2%)		11 (16.7%)	6 (14.0%)	3 (27.3%)		4 (21.1%)	9 (15.5%)	7 (16.3%)	
II: n (%)	36 (50.0%)	19 (51.4%)	4 (36.4%)		32 (48.5%)	24 (55.8%)	3 (27.3%)		8 (42.1%)	32 (55.2%)	19 (44.2%)	
III: n (%)	18 (25.0%)	10 (27.0%)	4 (36.4%)		16 (24.2%)	12 (27.9%)	4 (36.4%)		5 (26.3%)	14 (24.1%)	13 (30.2%)	
IV: n (%)	6 (8.3%)	2 (5.4%)	1 (9.1%)		7 (10.6%)	1 (2.3%)	1 (9.1%)		2 (10.5%)	3 (5.2%)	4 (9.3%)	
Grade III and IV				0.733				0.626				0.543
PN: n (%)	24 (33.3%)	12 (32.4%)	5 (45.5%)		23 (34.8%)	13 (30.2%)	5 (45.5%)		7 (36.8%)	17 (29.3%)	17 (39.5%)	

PN, peripheral neuropathy; [a]Pearson's Chi-squared tests; p-value < 0.05 is significant.

Table 5. Incidence of other toxicity grades distributed among the three haplotypes in each SNP

Toxicity	rs4148396 CC n=72	CT n=37	TT n=11	*P* [a]	rs3740066 CC n=66	CT n=43	TT n=11	*P* [a]	rs1885301 AA n=19	AG n=58	GG n=43	*P* [a]
Neutropenia				0.012*				0.073				0.385
I: n (%)	8 (11.1%)	2 (5.4%)	4 (36.4%)		6 (9.1%)	4 (9.3%)	4 (36.4%)		4 (21.1%)	5 (8.6%)	5 (11.6%)	
II: n (%)	47 (65.3%)	25 (67.6%)	2 (18.2%)		43 (65.2%)	28 (65.1%)	3 (27.3%)		8 (42.1%)	37 (63.8%)	29 (67.4%)	
III: n (%)	17 (23.6%)	9 (24.3%)	5 (45.5%)		17 (25.8%)	10 (23.3%)	4 (36.4%)		7 (36.8%)	15 (25.9%)	9 (20.9%)	
IV: n (%)	0 (0.0%)	1 (2.7%)	0 (0.0%)		0 (0.0%)	1 (2.3%)	0 (0.0%)		0 (0.0%)	1 (1.7%)	0 (0.0%)	
Vomiting				0.258				0.145				0.231
I: n (%)	13 (18.1%)	7 (18.9%)	5 (45.5%)		11 (16.7%)	10 (23.3%)	4 (36.4%)		5 (26.3%)	12 (20.7%)	8 (18.6%)	
II: n (%)	45 (62.5%)	22 (59.5%)	3 (27.3%)		42 (63.6%)	24 (55.8%)	4 (36.4%)		7 (36.8%)	35 (60.3%)	28 (65.1%)	
III: n (%)	10 (13.9%)	7 (18.9%)	3 (27.3%)		8 (12.1%)	9 (20.9%)	3 (27.3%)		7 (36.8%)	8 (13.8%)	5 (11.6%)	
IV: n (%)	4 (5.6%)	1 (2.7%)	0 (0.0%)		5 (7.6%)	0 (0.0%)	0 (0.0%)		0 (0.0%)	3 (5.2%)	2 (4.7%)	
Diarrhea				0.678				0.863				0.859
I: n (%)	17(23.6%)	9 (24.3%)	5 (45.5%)		15 (22.7%)	12 (27.9%)	4 (36.4%)		7 (36.8%)	13 (22.4%)	11 (25.6%)	
II: n (%)	34 (47.2%)	19 (51.4%)	4 (36.4%)		32 (48.5%)	21 (48.8%)	4 (36.4%)		8 (42.1%)	29 (50.0%)	20 (46.5%)	
III: n (%)	14 (19.4%)	8 (21.6%)	2 (18.2%)		13 (19.7%)	8 (18.6%)	3 (27.3%)		4 (21.1%)	11 (19.0%)	9 (20.9%)	
IV: n (%)	7 (9.7%)	1 (2.7%)	0 (0.0%)		6 (9.1%)	2 (4.7%)	0 (0.0%)		0 (0.0%)	5 (8.6%)	3 (7.0%)	

* Indicates significance; [a]Fisher's exact test; p value < 0.05 is significant.

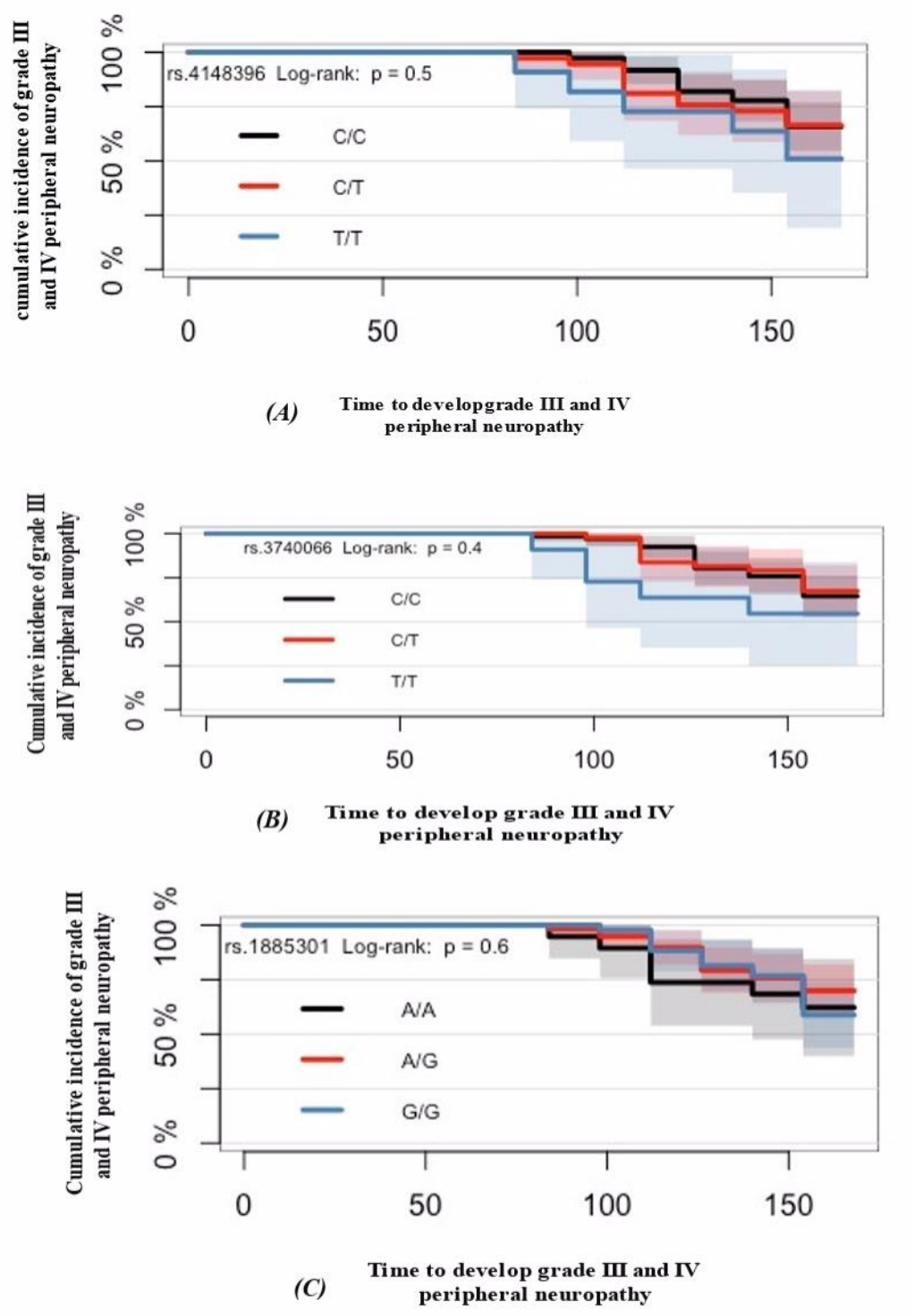

Fig.1. Kaplan-Meier curve to show the probability of developing grade III and IV peripheral neuropathy. A: for rs4148396 C>T, B: for rs3740066 C>T, C: for rs1885301 G >A.

4. Discussion

Oxaliplatin-induced PN may be linked to genetic polymorphisms associated with oxaliplatin uptake and metabolism, with the ABCC2 gene being one of the regulators of oxaliplatin accumulation in cells **[12]**. This study aimed to investigate the association between ABCC2 gene polymorphisms rs1885301 G>A, rs4148396 C>T, and rs3740066 C>T and the incidence and severity of OXAIPN in patients with gastrointestinal cancer.

According to the National Center for Biotechnology Information (NCBI)'s alfa project, the MAF of rs1885301 in the African population was 0.4, which was comparable to that reported in the present study **[18]**. Similarly, the MAF of rs4148396 was 0.2 in the present study, which was close to the frequency of 0.27 reported in the African population **[19]**. However, the MAF of rs3740066 was reported to be 0.3 in the current study which was different from that reported in the African population 0.2 but similar to that reported in Europe 0.3 **[20]**.

The overall incidence of OXAIPN was estimated to range from 40 to 98%, with clinically significant grades III and IV occurring in approximately 15% of cases **[21, 22]**. In the present investigation, all patients were found to have PN, with the incidence of grades III and IV being 34.2%. This was significantly greater than what Cecchin and colleagues found in their trial, where 83% of eligible patients had OXAIPN during treatment, but only 7% of these patients got grade III and IV PN **[5].** Similarly, 69.3% of patients in another trial had OXAIPN, but only 13% of them were grade III and IV **[23]**. This could be related to the fact that patients in these earlier studies only had three months of oxaliplatin-based chemotherapy, whereas patients in the current research received treatment for six months.

There is no connection between the three investigated SNPs of the ABCC2 gene and the incidence or time to acquire grade III and IV OXAIPN based on the existing data. Similarly, In an observational analysis of FOLFOX4-treated colorectal cancer patients, the same SNPs were not linked with the occurrence or severity of PN **[23]**. Contrary, in an observational analysis of FOLFOX4-treated colorectal cancer patients, the same SNPs were significantly associated with high-risk PN. However, this previous study was limited to colorectal cancer patients, and the authors deemed a p-value of less than 0.1 to be statistically significant **[5]**.

A regimen involving oxaliplatin can cause grade III and IV neutropenia at a rate between 37% to 56% **[22]**. Neutropenia grades III and IV were observed in 26.6 percent of participants, which was lower than Cecchin's study, which reported that 38 percent of their patients developed grades III and IV neutropenia, and comparable to Ruzzo's study, which reported grade III and IV in approximately 29 percent of participants **[5, 23]**. According to the present study, haplotype TT of ABCC2 rs4148396 C > T was related to a more severe form of neutropenia than haplotype CT+CC. However, the prevalence of haplotype TT was low, and additional research is required to clarify these findings. Contrarily, recent research evaluating the influence of this SNP on neutropenia found no connection. **[5, 23]**. Similar to earlier research, the other two SNPs, rs3740066 C > T and rs1885301 G > A, showed no connection with the incidence of neutropenia **[5, 23]**. Similarly, the SNP rs3740066 C > T was previously examined in non-small cell lung cancer patients taking irinotecan and cisplatin, with similar results indicating no effect on the incidence of neutropenia **[24]**.

The current investigation found no connection between the three SNPs with diarrhea

and vomiting incidence. Cecchin and his colleagues, as well as Ruzo and his colleagues, reported comparable findings **[5, 23]**. Han and colleagues discovered that the ABCC2 rs3740066 C>T genotype CC was associated with an increased incidence of diarrhea in non-small cell lung cancer patients receiving irinotecan and cisplatin **[24]**.

Limitations

The study was limited by the small size of the sample, short follow-up period, and contained different forms of cancer histology and grade.

Conclusion

In the Egyptian population, the MAF of the SNPs rs1885301 G>A, rs4148396 C>T, and rs3740066 C>T of the ABCC2 gene were comparable to that observed in Europe and Africa. No connection was found between the investigated SNPs of ABCC2 rs4148396 C>T, rs3740066 C>T, and rs1885301 G>A and the occurrence of PN, diarrhea, and vomiting. According to the rs3740066 C>T, the incidence of neutropenia was substantially higher in the TT haplotype than in the CT+CC haplotype.

Recommendations

To corroborate the results of the current investigation and specifically to analyze the link between neutropenia and the SNP rs4148396 C>T, a study with a larger sample size is advised.

List of abbreviations

ABCC2, ATP-Binding Cassette C2; SNPs, Single nucleotide polymorphisms; OXAIPN, oxaliplatin-induced peripheral neuropathy; PN, peripheral neuropathy; DRG, dorsal root ganglia; ABC, ATP-binding cassette; MDR, multidrug resistance; MRP2, multidrug resistance protein 2; ANC, Absolute Neutrophil Count; eGFR, Estimated Glomerular Filtration Rate; ALT, Alanine Transaminase Test; AST, aspartate aminotransferase; BSA, Body surface area; EDTA, Ethylenediaminetetraacetic acid; MAF, Minor allele frequency; NCI-CTCAE, National Cancer Institute's Common Toxicity Criteria for Adverse Event; IQR, interquartile range; NCBI, National Center for Biotechnology Information.

Declarations

Consent to publish

All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study was done following the 1964 Declaration of Helsinki and its 2013 revision. The research protocol with approval number was amended and authorized by the research ethics committee for experimental and clinical studies at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt: Master (Eneric – ASU .2020 – 297). ClinicalTrials.gov registration number: NCT05494320.

Availability of data and material

All data generated or analyzed during this study are included in this published article in the main manuscript.

Conflict of Interest

The authors assert that there are no conflicts of interest.

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Authors Contribution

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Sara Mohamed Abdel Aziz and May Ahmed Shawki. The first draft of the manuscript was written by Sara Mohamed Abdel Aziz and May Ahmed Shawki and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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