ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ANNALS OF ONCOLOGY driving innovation in oncology

ORIGINAL ARTICLE

Large-scale prospective genome-wide association study of oxaliplatin in stage II/III colon cancer and neuropathy

M. Kanai[1*], T. Kawaguchi[2], M. Kotaka[3], D. Manaka[4], J. Hasegawa[5], A. Takagane[6], Y. Munemoto[7], T. Kato[8], T. Eto[9], T. Touyama[10], T. Matsui[11], K. Shinozaki[12], S. Matsumoto[13], T. Mizushima[14], M. Mori[15], J. Sakamoto[16,17], A. Ohtsu[18], T. Yoshino[18], S. Saji[16] & F. Matsuda[2]

[1]Department of Therapeutic Oncology, Graduate School of Medicine, Kyoto University, Kyoto; [2]Center for Genomic Medicine, Graduate School of Medicine, Kyoto University, Kyoto; [3]Gastrointestinal Cancer Center, Sano Hospital, Kobe; [4]Department of Surgery, Gastrointestinal Center, Kyoto-Katsura Hospital, Kyoto; [5]Department of Surgery, Osaka Rosai Hospital, Osaka; [6]Department of Surgery, Hakodate Goryoukaku Hospital, Hokkaido; [7]Department of Surgery, Fukui Ken Saiseikai Hospital, Fukui; [8]Department of Surgery, Kansai Rosai Hospital, Hyogo; [9]Department of Gastroenterology, Tsuchiura Kyodo General Hospital, Ibaraki; [10]Department of Surgery, Nakagami Hospital, Okinawa; [11]Department of Gastroenterological Surgery, Aichi Cancer Center Aichi Hospital, Aichi; [12]Division of Clinical Oncology, Hiroshima Prefectural Hospital, Hiroshima; [13]Department of Real World Data Research and Development, Graduate School of Medicine, Kyoto University, Kyoto; [14]Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, Osaka; [15]Department of Surgery and Science, Kyushu University, Fukuoka; [16]Japanese Foundation for Multidisciplinary Treatment of Cancer, Tokyo; [17]Tokai Central Hospital, Kakamigahara; [18]Department of Gastroenterology and Gastrointestinal Oncology, National Cancer Center Hospital East, Chiba, Japan

Available online 12 August 2021

Background: The severity of oxaliplatin (L-OHP)-induced peripheral sensory neuropathy (PSN) exhibits substantial interpatient variability, and some patients suffer from long-term, persisting PSN. To identify single-nucleotide polymorphisms (SNPs) predicting L-OHP-induced PSN using a genome-wide association study (GWAS) approach.

Patients and methods: A large prospective GWAS including 1379 patients with stage II/III colon cancer who received L-OHP-based adjuvant chemotherapy (mFOLFOX6/CAPOX) under the phase II (JOIN/JFMC41) or the phase III (ACHIVE/JFMC47) trial. Firstly, GWAS comparison of worst grade PSN (grade 0/1 versus 2/3) was carried out. Next, to minimize the impact of ambiguity in PSN grading, extreme PSN phenotypes were selected and analyzed by GWAS. SNPs that could predict time to recovery from PSN were also evaluated. In addition, SNPs associated with L-OHP-induced allergic reactions (AR) and time to disease recurrence were explored.

Results: No SNPs exceeded the genome-wide significance ($P < 5.0 \times 10^{-8}$) in either GWAS comparison of worst grade PSN, extreme PSN phenotypes, or time to recovery from PSN. An association study focusing on AR or time to disease recurrence also failed to reveal any significant SNPs.

Conclusion: Our results highlight the challenges of utilizing SNPs for predicting susceptibility to L-OHP-induced PSN in daily clinical practice.

Key words: oxaliplatin, peripheral sensory neuropathy, genome-wide association study, pharmacogenomics, colon cancer

INTRODUCTION

Oxaliplatin (L-OHP), a third-generation diaminocyclohexane platinum compound, is used as a key chemotherapeutic agent for colorectal cancer. Peripheral sensory neuropathy (PSN) is a common dose-limiting toxicity associated with L-OHP-based chemotherapy.[1] Importantly, the severity of PSN exhibits substantial interpatient variability and some patients experience long-term, persisting PSN after the discontinuation of L-OHP.[2-4] Over the past two decades, many groups, us included, have attempted to identify genetic markers that could predict L-OHP-induced PSN using pharmacogenomics approaches.[5-13] Although multiple candidate single-nucleotide polymorphisms (SNPs) have been identified, no genomic markers have been introduced into daily clinical practice due to the lack of replication across the studies. Common problems associated with previous pharmacogenomics studies included small sample size, overrated *P* values without considering correction for multiple testing, retrospective study design, and lack of adjustment for the total dose of L-OHP.[13,14] Moreover, the effect size of genetic variations can be different between ethnicities, in part due to differences in their allele

*Correspondence to: Dr Masashi Kanai, Department of Therapeutic Oncology, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan. Tel: +81-75-751-4349; Fax: +81-75-751-4352

E-mail: kanai@kuhp.kyoto-u.ac.jp (M. Kanai).

frequencies.[15] To circumvent these drawbacks, we conducted a large-scale, prospective, genome-wide association study (GWAS) as a part of the phase II (JOIN/JFMC41) and phase III (ACHIVE/JFMC47) adjuvant clinical trial. The JOIN/JFMC41 trial enrolled 893 patients with stage II/III colon cancer,[16,17] while the ACHIVE/JFMC47 trial enrolled 1313 patients with stage III colon cancer.[4,18] Both trials provided adequate clinical information and were ideal platforms for GWAS targeting L-OHP. The primary objective of this GWAS was to identify SNPs associated with L-OHP-induced PSN. In addition to L-OHP-induced PSN, the SNPs associated with L-OHP-induced allergic reaction (AR) or time to disease recurrence were explored.

PATIENTS AND METHODS

Patients

Patients who underwent L-OHP-based adjuvant chemotherapy under clinical trials (JOIN/JFMC41 or ACHIVE/JFMC47) were enrolled in this preplanned prospective GWAS. JOIN/JFMC41 was a phase II trial and examined the safety of 12 cycles of modified FOLFOX6 (mFOLFOX6) in 893 patients with stage II/III colon cancer.[16,17] In contrast, ACHIVE/JFMC47, which was one of the six phase III trials of the International Duration Evaluation of Adjuvant Chemotherapy (IDEA),[19] tested 3 versus 6 months of mFOLFOX6 or capecitabine plus oxaliplatin (CAPOX) adjuvant chemotherapy and enrolled 1313 patients with stage III colon cancer.[4,18] In the JOIN/JFMC41 trial, all patients received the same protocol treatment of mFOLFOX6 (85 mg/m^2 L-OHP, 200 mg/m^2 l-leucovorin, 400 mg/m^2 5-fluorouracil bolus, and 2400 mg/m^2 5-fluorouracil infusion) for 6 months at 2-week intervals. In the ACHIVE/JFMC47 trial, patients were randomly assigned to receive 3 or 6 months of mFOLFOX6 or CAPOX (130 mg/m^2 L-OHP on day 1 followed by oral 1000 mg/m^2 capecitabine twice daily on days 1-14 at 3-week intervals). The selection between mFOLFOX6 and CAPOX was at the discretion of treating physicians. L-OHP, 5-fluorouracil, and capecitabine doses were adjusted according to the criteria predefined in each study protocol. For the present GWAS, 486 of the 882 patients enrolled in the JOIN/JFMC41 trial between November 2010 and March 2012 and 893 of the 1313 patients enrolled in the ACHIVE/JFMC47 trial between August 2012 and June 2014 provided written informed consent. In total, 14 patients were excluded from the final analysis due to the following reasons: study ineligibility ($n = 7$), consent withdrawal ($n = 3$), no administration of protocol treatment ($n = 3$), and no DNA sample ($n = 1$). In addition, one patient was excluded due to low call rate. Therefore, 1364 patients were analyzed in this GWAS (Figure 1A).

Clinical evaluation of L-OHP-induced PSN and AR

L-OHP-induced PSN and AR were evaluated by investigators in each institution. In the JOIN/JFMC41 trial, PSN was evaluated according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) versions 1.0 and 2.0 and the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 as previously reported.[16] In the ACHIVE/JFMC47 trial, CTCAE version 4.0 was adopted for PSN grading.[4,18] AR was graded according to the CTCAE versions 3.0 and 4.0 in the JOIN/JFMC41 and ACHIVE/JFMC47 trials, respectively. Since essential elements of PSN and AR grading remained unchanged between CTCAE version 3.0 and 4.0, we considered that the data from the two clinical trials were comparable. Data were collected using an electronic data capture system, and central monitoring was carried out in both trials.

Genotyping and quality control

Genomic DNA was extracted from peripheral blood samples according to a standard phenol-chloroform extraction method and stored at −20 °C until use. Two BeadChip DNA arrays, CoreExome and Asian Screening Array (Illumina, San Diego, CA), were used for genotyping in all patients. We confirmed that no samples exceeded a discordant rate of 0.1% by comparing the genotypes from two SNP arrays that overlapped 114 076 SNPs. Discordant genotypes among the overlapped SNPs were set as missing. We confirmed that there were neither outliers of the Japanese cluster according to the principal component analysis using the genotype data from the 1000 Genome Project (Phase III, ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/) nor first-degree relatedness. We also confirmed that the genotype call rate of overall chromosomes in each sample was >95%, although one sample was removed from the analysis due to the low call rate (<90%) in a particular chromosome. For the variant in the chromosome X, we initially carried out the analysis for the males and females separately and dose of the effective allele for the male was set to 1. Then, using the Mantel−Haenszel method, we merged the analysis data for each sex. Next, we excluded SNPs with the following features from the analysis of the final set of 1 086 831 SNPs: SNPs with a call rate <99% ($n =$ 65 520), SNPs with minor allele frequencies <0.01 ($n =$ 409 436), and SNPs with significant deviation ($P < 1.0 \times 10^{-6}$) from the Hardy−Weinberg equilibrium ($n =$ 587). Using the obtained genotypes for 611 288 SNPs, we carried out genotype imputation using the SHAPEIT2[20] and Minimac4[21] tools, by referencing the 21 834 763 SNPs of in-house imputation reference panel consisting of 3135 Japanese whole genome sequencing (unpublished data). For X chromosome imputation, we split the data according to sex following the instructions of the tools. We excluded 12 239 104 imputed SNPs with rsq values of <0.8 and 3 182 756 SNPs with minor allele frequencies of <0.01. Finally, we obtained the genotype dataset for 1364 patients and included 6 412 903 SNPs for subsequent association analyses (Supplementary Figure S1, available at https://doi.org/10.1016/j.annonc.2021.08.1745).

Study cohort for L-OHP-induced PSN

Patients with worst PSN grades of 0 or 1 who were randomly assigned to the 3-month treatment group in the

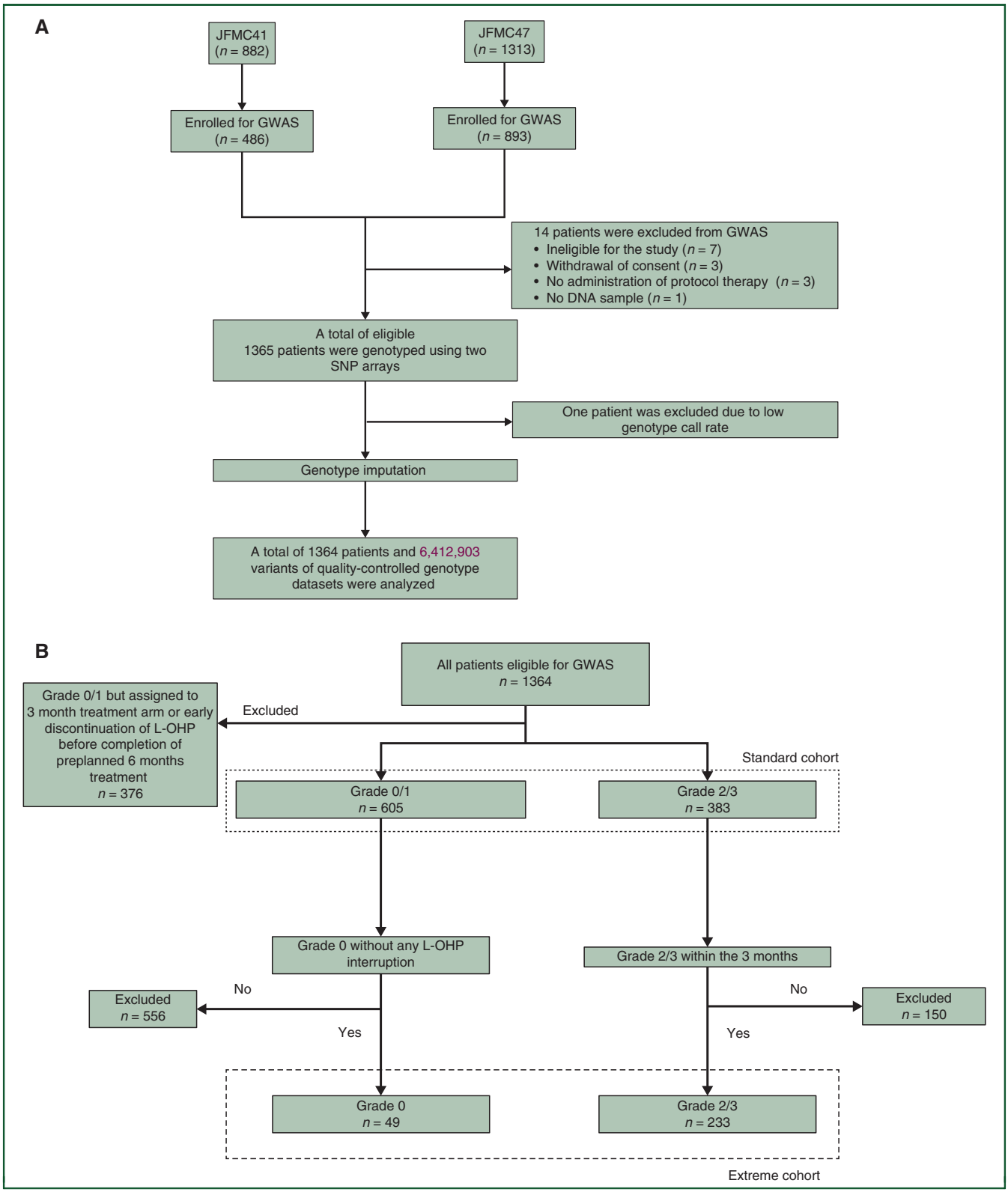

Figure 1. (A) Study flow. (B) Peripheral sensory neuropathy study cohort. (C) Allergic reactions study cohort.
GWAS, genome-wide association study; L-OHP, oxaliplatin; SNP, single-nucleotide polymorphism.

ACHIVE/JFMC47 trial or discontinued L-OHP before completing the preplanned 6-month of adjuvant treatment were excluded from GWAS for PSN ($n = 376$), because low PSN grade in these patients could simply be due to lower cumulative L-OHP dose. Therefore, 605 patients with grade 0/1 PSN were compared to 383 patients with grade 2/3 PSN (Figure 1B). Next, extreme phenotypes of PSN were selected for GWAS comparison to minimize the impact of ambiguity in PSN grading. Patients who discontinued L-OHP early due to grade 2/3 PSN ($n = 233$) were selected as extremely

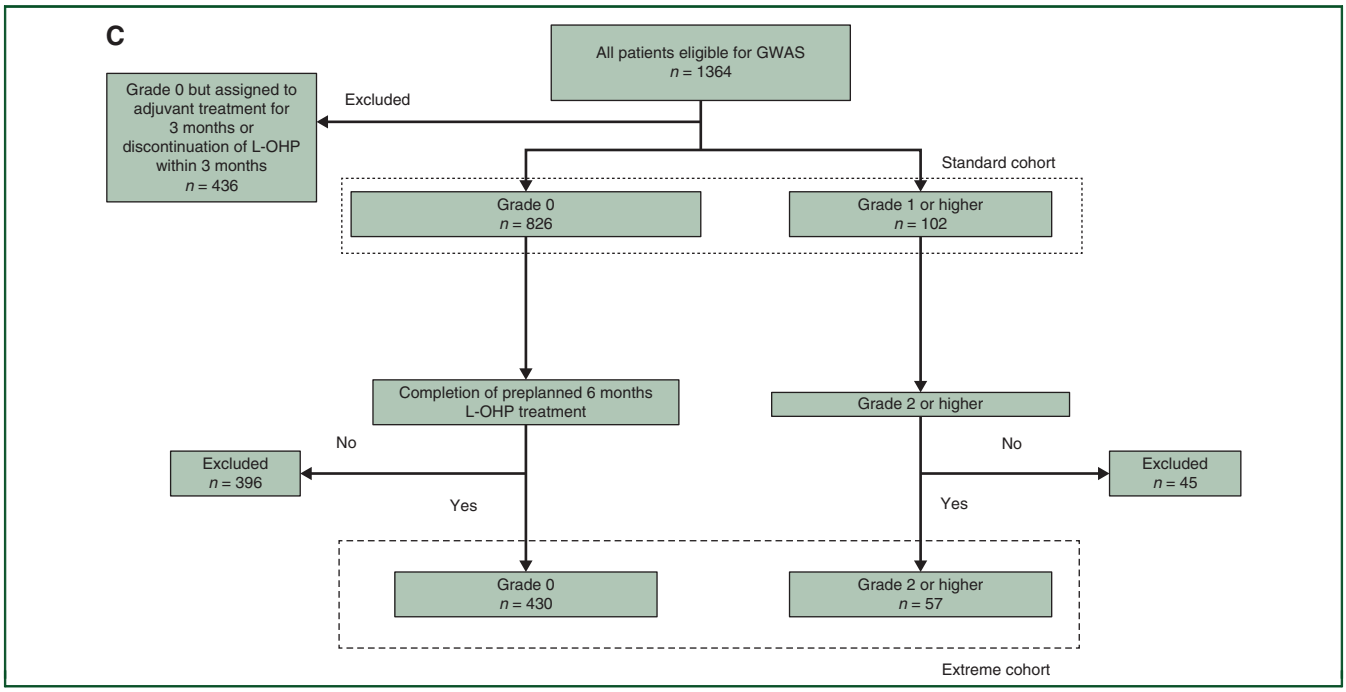

Figure 1. Continued.

vulnerable PSN phenotypes and were compared to those who maintained the status of grade 0 PSN after the completion of preplanned 6-month treatment without any dose reduction or delay of L-OHP ($n = 49$) (Figure 1B).

In both JOIN/JFMC41 and ACHIVE/JFMC47 trials, PSN grades were prospectively collected under the protocol for up to 3 years after adjuvant treatment initiation. After completion of the adjuvant treatment, PSN was evaluated every 3 months unless patients experienced any events of recurrence, secondary cancer, or death. For the analysis of time to recovery from PSN, the intervals were determined from the date of adjuvant treatment completion to the date of recovery from the worst-grade PSN during the treatment. Patients who did not develop PSN (grade 0) during adjuvant treatment were excluded from the analysis ($n = 228$).

Study cohort for L-OHP-induced AR

A total of 436 patients who did not develop AR but were randomly assigned to the 3-month treatment group or discontinued L-OHP within 3 months for any reason other than L-OHP-induced AR were excluded from GWAS for AR. This is because the risk of AR is lower during the first 3 months of L-OHP administration.[19] Therefore, 826 patients with grade 0 AR were compared to 102 patients with grade 1 or higher AR (Figure 1C). Next, 57 patients who developed grade 2 or higher AR were selected as extreme phenotypes and compared to 430 patients who did not develop AR during the preplanned 6 months of treatment without L-OHP discontinuation (Figure 1C).

Time to disease recurrence analysis

The intervals were determined from the date of adjuvant treatment initiation to disease recurrence. Patients not experiencing disease recurrence were censored at the last follow-up.

GWAS

Logistic regression was carried out based on allele dosage using plink-2.0[22] with top two principal components (PCs) derived from the genotype dataset, regimen (mFOLFOX6 versus CAPOX), trial (JOIN/JFMC41 versus ACHIVE/JFMC47), and preplanned treatment period (3 versus 6 months) as covariates for statistical analysis of the GWAS data. In the analysis of time to recovery from PSN, Cox-hazard regression was carried out using gwasurvivr and the same covariates as the logistic regression analysis were used as covariates. In the analysis of disease recurrence, Cox hazard regression was carried out using gwasurvivr and the top two PCs, T-stage, N-stage, lymph node ratio, and histology type were applied as covariates (TNM classification). Manhattan and quantile-quantile (QQ) plots were obtained by qqman, and genotype frequency was determined by plink-2.0 based on the default hard call threshold from allelic dosage. Genomic inflation due to residual population stratification was assessed by the inflation factor.[23] Genome-wide significance was set as a P value of 5.0×10^{-8} based on Bonferroni's correction for multiple testing. Additionally, a P value of 1.0×10^{-5} was set as a threshold of suggestive associations. In significant or suggestive loci, the lead SNP was determined as the SNP with the lowest P value within that 1-Mb region.

Ethics

The study protocols were approved by the institutional review boards of all participating institutions, and all patients

https://doi.org/10.1016/j.annonc.2021.08.1745

Table 1. Patients' characteristics

Trial	JOIN/JFMC41	ACHIVE/JFMC47			
Regimen	mFOLFOX	mFOLFOX6		CAPOX	
Treatment period (months)	6	6	3	6	3
Sample number	481	120	120	322	321
Completion of protocol treatment, *n* (%)	269 (56)	52 (43)	98 (82)	151 (47)	284 (88)
Full completion of protocol treatment,[a] *n* (%)	25 (5)	4 (3)	29 (24)	9 (3)	96 (30)
Median age, years (range)	64 (21-83)	66 (34-82)	66 (31-85)	64 (36-85)	64 (34-83)
Sex, *n* (%)					
Male	261 (54)	59 (49)	63 (53)	163 (51)	169 (53)
Female	220 (46)	61 (51)	57 (47)	159 (49)	152 (47)
PSN, *n* (%)					
Gr0	56 (12)	13 (11)	27 (23)	55 (17)	81 (25)
Gr1	258 (54)	70 (58)	80 (66)	153 (48)	188 (59)
Gr2	140 (29)	35 (29)	12 (10)	99 (30)	48 (15)
Gr3	27 (6)	2 (2)	1 (1)	15 (5)	4 (1)
Allergic reactions, *n* (%)					
Gr0	410 (85)	102 (85)	117 (97)	309 (96)	318 (99)
Gr1	35 (7)	4 (3)	2 (2)	4 (1)	1 (0)
Gr2	28 (6)	12 (10)	1 (1)	6 (2)	2 (1)
≥Gr3 *n*= (%)	8 (2)	2 (2)	0 (0)	3 (1)	0 (0)

CAPOX, capecitabine plus oxaliplatin; mFOLFOX, modified FOLFOX; PSN, peripheral sensory neuropathy.
[a] No dose reduction or delay of protocol treatment.

provided written informed consent for the use of genomic and clinical data for research purposes.

RESULTS

Patient characteristics

Of a total of 1379 patients enrolled in the GWAS analysis, data from 1364 patients were analyzed (Figure 1A). The patient characteristics are summarized in Table 1. Age, sex, and incidence of worst-grade PSN during adjuvant treatment were comparable to those reported in the IDEA study.[19]

GWAS for L-OHP-induced PSN

In the standard PSN cohort (Figure 1B), the comparison of patients with grade 0/1 PSN to those with grade 2/3 PSN failed to identify any genome-wide significant SNPs. The lead SNPs with suggestive associations ($P < 1.0 \times 10^{-5}$) are listed in Table 2 Standard cohort. Next, we carried out a GWAS using the cohort with extreme PSN phenotypes (Figure 1B). However, no SNP exceeded the genome-wide significance threshold. The lead SNPs with suggestive associations are listed in Table 2 Extreme cohort. All SNPs with suggestive association are listed in Supplementary Tables S1 and S2, available at https://doi.org/10.1016/j.annonc.2021.08.1745 and the Manhattan and QQ plots are shown in Supplementary Figure S2, available at https://doi.org/10.1016/j.annonc.2021.08.1745.

GWAS for time to recovery from PSN

The probabilities of time to recovery from PSN are shown in Supplementary Figure S3, available at https://doi.org/10.1016/j.annonc.2021.08.1745. The GWAS was carried out to explore SNPs that could predict recovery from PSN; no SNPs however, exceeded the genome-wide significance threshold (Table 3). All SNPs with suggestive associations are listed in Supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2021.08.1745 and the Manhattan and QQ plots are shown in Supplementary Figure S4, available at https://doi.org/10.1016/j.annonc.2021.08.1745.

GWAS for L-OHP-induced AR

In the standard AR cohort (Figure 1C), the comparison of patients with grade 0 AR to those with grade 1 or higher AR failed to identify any genome-wide significant SNPs. Next, we carried out a GWAS using the cohort with extreme AR phenotypes (Figure 1C). No SNPs however, exceeded the genome-wide significance threshold. All SNPs with suggestive associations are listed in Supplementary Tables S4 and S5, available at https://doi.org/10.1016/j.annonc.2021.08.1745 and the Manhattan and QQ plots are shown in Supplementary Figure S5, available at https://doi.org/10.1016/j.annonc.2021.08.1745.

GWAS for time to disease recurrence

The probabilities of disease recurrence are shown in Supplementary Figure S6, available at https://doi.org/10.1016/j.annonc.2021.08.1745. The GWAS was carried out to explore SNPs that could predict the disease recurrence; however, no SNPs exceeded the genome-wide significance threshold. All SNPs with suggestive associations are listed in Supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2021.08.1745 and the Manhattan and QQ plots are shown in Supplementary Figure S7, available at https://doi.org/10.1016/j.annonc.2021.08.1745.

Table 2. Suggestive lead SNPs associated with oxaliplatin-induced PSN

Gene	rsID	chr	pos	ref/alt	Genotype count, allele frequency		*P* value	OR (95% CI)
					Grade 2/3	Grade 0/1		
Standard cohort								
LINC00901,LINC02024	rs76299149	3	116973739	G/A	0.25	0.17	5.6×10^{-6}	1.72 (0.12-1.36)
GABRB1	rs6829206	4	47052606	A/T	0.9	0.83	2.7×10^{-6}	2.14 (0.16-1.56)
NONE,LINC00290	rs12501594	4	181526148	C/T	0.049	0.021	3.6×10^{-6}	3.09 (0.24-1.92)
DTNBP1,MYLIP	rs9476901	6	15731306	T/C	0.61	0.51	8.4×10^{-6}	1.57 (0.10-1.29)
RECK	rs112917429	9	36101017	G/A	0.098	0.046	4.3×10^{-6}	2.33 (0.18-1.62)
MEX3B,LINC01583	rs7169642	15	82360813	C/T	0.51	0.62	3.3×10^{-6}	0.61 (0.10-0.50)
LINC00907	rs17610383	18	39892281	G/A	0.11	0.06	1.6×10^{-6}	2.3 (0.17-1.66)
Gene	**rsID**	**chr**	**pos**	**ref/alt**	**Genotype count, allele frequency**		***P* value**	**OR (95% CI)**
					Grade 2/3	Grade 0		
Extreme cohort								
LOC399715,PRKCQ	rs2181623	10	6391491	C/T	0.92	0.74	8.1×10^{-6}	4.86 (0.35-2.42)
ATP7B	rs117036130	13	52551794	T/C	0.078	0.25	5.1×10^{-6}	0.20 (0.34-0.10)

Odds ratios are calculated for alternative allele.
chr, chromosome; OR, odds ratio; pos, position; PSN, peripheral sensory neuropathy; ref/alt, reference and alternative allele at the position; SNP, single-nucleotide polymorphism.

DISCUSSION

Chemotherapy-induced PSN is one of the most serious non-hematological adverse events in cancer chemotherapy.[14] Cumulative drug dose is positively correlated with the incidence and severity of PSN; since PSN grade greatly differs between patients however, genetic variability is proposed to contribute to the differential susceptibility to chemotherapy-induced PSN. To address this issue, pharmacogenomics approaches have been utilized over the past two decades. Followed by candidate gene approaches, comprehensive genome-wide scan using GWAS has become a popular tool to explore causal genetic variants of chemotherapy-induced PSN.[11,24-32] Several candidate genetic markers for L-OHP induced PSN have been proposed so far.[5-7,11,12,33,34] In our previous study, we tested 12 SNPs that were demonstrated to be significantly associated with L-OHP-induced PSN in at least two independent cohorts in the literature, but none of these markers were replicated in the JOIN/JFMC41 dataset.[13] Common problems associated with interpreting the results of published pharmacogenomics studies include inadequate sample size, overrated *P* values without consideration of correction for multiple testing, inaccurate information on PSN due to a retrospective study design, and lack of adjustment for the total dose of anticancer drugs.[13,14] The current GWAS was designed to overcome these drawbacks. Firstly, this study enrolled a total of 1379 patients with stage II/III colon cancer who received adjuvant mFOLFOX6/CAPOX under clinical trials and the administration and dose adjustment of L-OHP were carried out according to predefined criteria. In addition, all patients were confirmed to be of Japanese ancestry by genotyping; therefore, the study results were not affected by racial diversity, which can sometimes complicate GWAS findings.[15] To the best of our knowledge, this is the largest pharmacogenomics study investigating L-OHP-induced PSN. Secondly, a rigorous genome-wide *P* value cut-off ($<5.0 \times 10^{-8}$) was adopted according to Bonferroni's correction for multiple testing. Thirdly, information on PSN was prospectively collected under the clinical trials. Finally, total L-OHP dose was incorporated into the analysis. Despite these efforts to address the caveats of earlier studies, no SNPs exceeded the genome-wide significance threshold in GWAS comparison for worst-grade PSN or time to recovery from PSN.

There are several potential explanations for these negative results. Firstly, PSN was graded based on the CTCAE, which is the gold standard for evaluating adverse events during chemotherapy, including PSN, in daily clinical practice; however, PSN grading involves a certain degree of ambiguity due to its subjective nature.[35,36] To minimize the impact of ambiguity in PSN grading, we selected extreme PSN phenotypes for comparison (Figure 1B), yet failed to identify SNPs exceeding the genome-wide significance threshold. Secondly, L-OHP-induced PSN may not be attributable to a limited number of SNPs with a large effect size. In general, SNPs responsible for specific drug toxicities have a larger effect size than disease associated SNPs.[15] For example, UDP-glucuronosyltransferase (UGT) 1A1 polymorphism is now commonly used for predicting the risk of irinotecan-associated neutropenia and its odds ratio was reported to be >5.0 in the original pharmacogenomics study.[37] If unknown SNPs associated with L-OHP-induced PSN with a similar effect size actually exist under 20% of allele frequency, the sample size of our study would have to be of >80% power to detect them; if numerous SNPs with small effect sizes are involved in the susceptibility to L-OHP-induced PSN however, a larger sample size would be required. Unlike traditional disease-associated GWAS however, it is unrealistic to enroll >10 000 cases with similar background characteristics who receive the same treatment for pharmacogenomics GWAS. Finally, SNPs that are not covered by the current GWAS analysis may play a pivotal role in the development of L-OHP-induced PSN; we consider that this possibility is low however, because we used two

Table 3. Suggestive lead SNPs associated with time to recovery from PSN

Gene	rsID	chr	pos	ref/alt	Allele frequency		*P* value	Hazard ratio (95% CI)
					Recovered	Not recovered		
DCDC2C	rs17018026	2	3836760	G/A	0.49	0.39	3.1×10^{-7}	1.31 (1.19-1.45)
CACNB4	rs146474026	2	152897870	C/T	0.037	0.011	2.7×10^{-7}	2.03 (1.58-2.62)
LMBRD1,COL19A1	rs62407462	6	70515105	T/C	0.65	0.58	9.9×10^{-6}	1.28 (1.16-1.42)
LOC101929297	rs35925426	6	166658945	G/A	0.32	0.41	5.4×10^{-7}	0.75 (0.68-0.83)
ARL4A,ETV1	rs62447871	7	13274596	A/T	0.36	0.24	5.1×10^{-6}	1.29 (1.17-1.44)
CSMD1	rs555118050	8	3098273	G/C	0.011	0.002	4.4×10^{-6}	3.06 (1.96-4.76)
LOC102725080,SGCZ	rs7009093	8	13590725	C/G	0.012	0.002	9.0×10^{-6}	2.83 (1.85-4.33)
MIR7641-2,LOC102724710	rs494821	8	93487821	C/G	0.73	0.80	4.6×10^{-6}	0.76 (0.69-0.85)
LINC00621,SGCG	rs7338725	13	23557542	C/G	0.34	0.24	4.6×10^{-7}	1.33 (1.20-1.48)
LINC02311,LINC02301	rs11159547	14	82955121	C/T	0.39	0.33	2.8×10^{-6}	1.29 (1.16-1.42)
GAS7	rs141164127	17	9850630	T/C	0.019	0.004	1.6×10^{-6}	2.12 (1.60-2.83)
MPP2	rs1642592	17	41969301	A/C	0.061	0.033	9.0×10^{-6}	1.61 (1.32-1.96)
CHD6,PTPRT	rs149840913	20	40681715	C/T	0.016	0.002	5.0×10^{-6}	2.56 (1.76-3.72)

Odds ratios are calculated for alternative allele.
chr, chromosome; pos, position; PSN, peripheral sensory neuropathy; ref/alt, reference and alternative allele at the position; SNPs, single-nucleotide polymorphisms.

different SNP arrays, one of which is developed based on the East-Asian SNP dataset.

In contrast to PSN, few pharmacogenomics studies examined L-OHP-induced AR. In the present study, we failed to identify the genetic variants associated with AR. In addition to the potential explanations mentioned above, human leukocyte antigen subtyping, which is beyond the target of SNP arrays employed in the present study, might play a pivotal role in the development of L-OHP-induced AR.

In the analysis of time to disease recurrence, no SNPs exceeded the genome-wide significance threshold. A recent GWAS study identified several genetic markers predicting early metastasis in a cohort of 379 patients with stage I—III colorectal cancer.[38] None of the SNPs however, were replicated in the present study (Supplementary Table S7, available at https://doi.org/10.1016/j.annonc.2021.08.1745). Considering the *P* value range (0.25-0.97) found in our cohort, it is unlikely that ethnic difference (Caucasian versus Asian) is the sole reason of inconsistent results between the two studies.

In summary, this large, prospective GWAS failed to identify novel genetic markers associated with L-OHP-induced PSN. The current results highlight the challenges of utilizing SNPs to predict L-OHP-induced PSN.

ACKNOWLEDGEMENTS

We thank the patients who participated in this study, and their families, and the physicians and medical staff of the 100 institutions registered in this study. We also thank Yakult Honsha Co., Ltd. (Mr. Takahiro Nitta, Mr. Keiichi Shimizu, and Mr. Hiroshi Takizawa).

FUNDING

This work was supported by Yakult Honsha Co., Ltd. (no grant number).

DISCLOSURE

MKa reports honoraria from Chugai Pharmaceutical Co., Ltd. MKo reports honoraria from Chugai Pharmaceutical Co., Ltd., Yakult Honsha Co., Ltd., and Takeda Pharmaceutical Company Limited. JH reports speaker bureau from Chugai Pharmaceutical Co., Ltd. and Yakult Honsha Co., Ltd. TK reports honoraria from Chugai Pharmaceutical Co., Ltd., Takeda Pharmaceutical Company Limited, Taiho Pharmaceutical Co., Ltd, ONO Pharmaceutical Co., Ltd. and research grant from Chugai Pharmaceutical Co., Ltd. and Takeda Pharmaceutical Company Limited. SM reports research grant from NTT Ltd., H.U. Group, and Canon Medical Systems. TM reports research grant from Takeda Pharmaceutical Company Limited, Shire Japan, Taiho Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Yakult Honsha Co., Ltd., Eli Lilly Japan, ONO Pharmaceutical Co., Ltd., Sanofi, Mitsubishi Tanabe, Kaken, Japan Blood Products Organization, Tsumura, Astellas, Pfizer Japan, Daiichi Sankyo, Merck Biopharma, and MSD. AO reports honoraria from BMS, ONO Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., and Chugai Pharmaceutical Co., Ltd. and research grant from BMS. TY reports honoraria from Taiho Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Eli Lilly Japan K.K., Bayer Yakuhin, Ltd., Merck Biopharma Co., Ltd., and Takeda Pharmaceutical Company Limited and research grant from MSD K.K., Ono Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Chugai Pharmaceutical Co., Ltd., Parexel International Inc., Sanofi K.K. Taiho Pharmaceutical Co., Ltd., Amgen K.K., and GlaxoSmithKline K.K. FM reports research grant from Yakult Honsha Co., Ltd. All other authors have declared no conflicts of interest.

REFERENCES

1. Grothey A. Oxaliplatin-safety profile: neurotoxicity. *Semin Oncol*. 2003;30(4 suppl 15):5-13.
2. Mols F, Beijers T, Lemmens V, van den Hurk CJ, Vreugdenhil G, van de Poll-Franse LV. Chemotherapy-induced neuropathy and its association with quality of life among 2- to 11-year colorectal cancer survivors: results from the population-based PROFILES registry. *J Clin Oncol*. 2013;31(21):2699-2707.
3. Pachman DR, Qin R, Seisler DK, et al. Clinical course of oxaliplatin-induced neuropathy: results from the randomized phase III trial N08CB (Alliance). *J Clin Oncol*. 2015;33(30):3416-3422.
4. Yoshino T, Yamanaka T, Oki E, et al. Efficacy and long-term peripheral sensory neuropathy of 3 vs 6 months of oxaliplatin-based adjuvant

chemotherapy for colon cancer: the ACHIEVE phase 3 randomized clinical trial. *JAMA Oncol*. 2019;5(11):1574-1581.
5. Lecomte T, Landi B, Beaune P, Laurent-Puig P, Loriot M-A. Glutathione S-transferase P1 polymorphism (Ile105Val) predicts cumulative neuropathy in patients receiving oxaliplatin-based chemotherapy. *Clin Cancer Res*. 2006;12(10):3050-3056.
6. Ruzzo A, Graziano F, Loupakis F, et al. Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFOX-4 chemotherapy. *J Clin Oncol*. 2007;25(10):1247-1254.
7. Goekkurt E, Al-Batran S-E, Hartmann JT, et al. Pharmacogenetic analyses of a phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil and leucovorin plus either oxaliplatin or cisplatin: a study of the arbeitsgemeinschaft internistische onkologie. *J Clin Oncol*. 2009;27(17):2863-2873.
8. Boige V, Mendiboure J, Pignon J-P, et al. Pharmacogenetic assessment of toxicity and outcome in patients with metastatic colorectal cancer treated with LV5FU2, FOLFOX, and FOLFIRI: FFCD 2000-05. *J Clin Oncol*. 2010;28(15):2556-2564.
9. Kanai M, Yoshioka A, Tanaka S, et al. Associations between glutathione S-transferase pi Ile105Val and glyoxylate aminotransferase Pro11Leu and Ile340Met polymorphisms and early-onset oxaliplatin-induced neuropathy. *Cancer Epidemiol*. 2010;34(2):189-193.
10. McLeod HL, Sargent DJ, Marsh S, et al. Pharmacogenetic predictors of adverse events and response to chemotherapy in metastatic colorectal cancer: results from North American Gastrointestinal Intergroup Trial N9741. *J Clin Oncol*. 2010;28(20):3227-3233.
11. Won H-H, Lee J, Park JO, et al. Polymorphic markers associated with severe oxaliplatin-induced, chronic peripheral neuropathy in colon cancer patients. *Cancer*. 2012;118(11):2828-2836.
12. Custodio A, Moreno-Rubio J, Aparicio J, et al. Pharmacogenetic predictors of severe peripheral neuropathy in colon cancer patients treated with oxaliplatin-based adjuvant chemotherapy: a GEMCAD group study. *Ann Oncol*. 2014;25(2):398-403.
13. Kanai M, Kawaguchi T, Kotaka M, et al. Large-scale prospective pharmacogenomics study of oxaliplatin-induced neuropathy in colon cancer patients enrolled in the JFMC41-1001-C2 (JOIN Trial). *Ann Oncol*. 2016;27(6):1143-1148.
14. Argyriou AA, Bruna J, Genazzani AA, Cavaletti G. Chemotherapy-induced peripheral neurotoxicity: management informed by pharmacogenetics. *Nat Rev Neurol*. 2017;13(8):492-504.
15. Mapes B, El Charif O, Al-Sawwaf S, Dolan ME. Genome-wide association studies of chemotherapeutic toxicities: genomics of inequality. *Clin Cancer Res*. 2017;23(15):4010-4019.
16. Kotaka M, Yoshino T, Oba K, et al. Initial safety report on the tolerability of modified FOLFOX6 as adjuvant therapy in patients with curatively resected stage II or III colon cancer (JFMC41-1001-C2: JOIN trial). *Cancer Chemother Pharmacol*. 2015;76(1):75-84.
17. Yoshino T, Kotaka M, Shinozaki K, et al. JOIN trial: treatment outcome and recovery status of peripheral sensory neuropathy during a 3-year follow-up in patients receiving modified FOLFOX6 as adjuvant treatment for stage II/III colon cancer. *Cancer Chemother Pharmacol*. 2019;84(6):1269-1277.
18. Kotaka M, Yamanaka T, Yoshino T, et al. Safety data from the phase III Japanese ACHIEVE trial: part of an international, prospective, planned pooled analysis of six phase III trials comparing 3 versus 6 months of oxaliplatin-based adjuvant chemotherapy for stage III colon cancer. *ESMO Open*. 2018;3(3):e000354.
19. Grothey A, Sobrero AF, Shields AF, et al. Duration of adjuvant chemotherapy for stage III colon cancer. *N Engl J Med*. 2018;378(13):1177-1188.
20. Delaneau O, Howie B, Cox AJ, Zagury J-F, Marchini J. Haplotype estimation using sequencing reads. *Am J Hum Genet*. 2013;93(4):687-696.
21. Das S, Forer L, Schönherr S, et al. Next-generation genotype imputation service and methods. *Nat Genet*. 2016;48(10):1284-1287.
22. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 2015;4:7.
23. Devlin B, Roeder K, Wasserman L. Genomic control, a new approach to genetic-based association studies. *Theor Popul Biol*. 2001;60(3):155-166.
24. Joerger M, Burgers SA, Baas P, et al. Germline polymorphisms in patients with advanced nonsmall cell lung cancer receiving first-line platinum-gemcitabine chemotherapy: a prospective clinical study. *Cancer*. 2012;118(9):2466-2475.
25. Magrangeas F, Kuiper R, Avet-Loiseau H, et al. A genome-wide association study identifies a novel locus for bortezomib-induced peripheral neuropathy in European patients with multiple myeloma. *Clin Cancer Res*. 2016;22(17):4350-4355.
26. Campo C, da Silva Filho MI, Weinhold N, et al. Bortezomib-induced peripheral neuropathy: a genome-wide association study on multiple myeloma patients. *Hematol Oncol*. 2018;36(1):232-237.
27. Baldwin RM, Owzar K, Zembutsu H, et al. A genome-wide association study identifies novel loci for paclitaxel-induced sensory peripheral neuropathy in CALGB 40101. *Clin Cancer Res*. 2012;18(18):5099-5109.
28. Leandro-Garcia LJ, Inglada-Perez L, Pita G, et al. Genome-wide association study identifies ephrin type A receptors implicated in paclitaxel induced peripheral sensory neuropathy. *J Med Genet*. 2013;50(9):599-605.
29. Wheeler HE, Gamazon ER, Wing C, et al. Integration of cell line and clinical trial genome-wide analyses supports a polygenic architecture of paclitaxel-induced sensory peripheral neuropathy. *Clin Cancer Res*. 2013;19(2):491-499.
30. Schneider BP, Li L, Radovich M, et al. Genome-wide association studies for Taxane-induced peripheral neuropathy in ECOG-5103 and ECOG-1199. *Clin Cancer Res*. 2015;21(22):5082-5091.
31. Hertz DL, Owzar K, Lessans S, et al. Pharmacogenetic discovery in CALGB (Alliance) 90401 and mechanistic validation of a VAC14 polymorphism that increases risk of docetaxel-induced neuropathy. *Clin Cancer Res*. 2016;22(19):4890-4900.
32. Diouf B, Crews KR, Lew G, et al. Association of an inherited genetic variant with vincristine-related peripheral neuropathy in children with acute lymphoblastic leukemia. *J Am Med Assoc*. 2015;313(8):815-823.
33. Chen Y-C, Tzeng C-H, Chen P-M, et al. Influence of GSTP1 I105V polymorphism on cumulative neuropathy and outcome of FOLFOX-4 treatment in Asian patients with colorectal carcinoma. *Cancer Sci*. 2010;101(2):530-535.
34. Hong J, Han S-W, Ham HS, et al. Phase II study of biweekly S-1 and oxaliplatin combination chemotherapy in metastatic colorectal cancer and pharmacogenetic analysis. *Cancer Chemother Pharmacol*. 2011;67(6):1323-1331.
35. Cavaletti G, Alberti P, Marmiroli P. Chemotherapy-induced peripheral neurotoxicity in the era of pharmacogenomics. *Lancet Oncol*. 2011;12(12):1151-1161.
36. Argyriou AA, Kyritsis AP, Makatsoris T, Kalofonos HP. Chemotherapy-induced peripheral neuropathy in adults: a comprehensive update of the literature. *Cancer Manag Res*. 2014;6:135-147.
37. Ando Y, Saka H, Ando M, et al. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res*. 2000;60(24):6921-6926.
38. Penney ME, Parfrey PS, Savas S, Yilmaz YE. A genome-wide association study identifies single nucleotide polymorphisms associated with time-to-metastasis in colorectal cancer. *BMC Cancer*. 2019;19(1):133.