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ORIGINAL ARTICLE

Single nucleotide polymorphism in *ABCG2* is associated with irinotecan-induced severe myelosuppression

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Irinotecan is an anti-neoplastic agent that is widely used for treating colorectal and lung cancers, but often causes toxicities such as severe myelosuppression and diarrhea. In this study, we performed a two-stage case–control association study for irinotecan-induced severe myelosuppression (grades 3 and 4). In the first stage, 23 patients who developed severe myelosuppression and 58 patients who did not develop any toxicity were examined for 170 single nucleotide polymorphisms (SNPs) in 14 genes involved in the metabolism and transport of irinotecan. A total of five SNPs were identified to show the possible association with severe myelosuppression ($P_{Fisher} < 0.01$) and were further examined in 7 cases and 20 controls in the second stage of the study. An intronic SNP, rs2622604, in *ABCG2* showed $P_{Fisher}=0.0419$ in the second stage and indicated a significant association with severe myelosuppression in the combined study ($P_{Fisher}=0.000237$; $P_{Corrected}=0.036$). Although only limited subjects were investigated, our results suggested that a genetic polymorphism in *ABCG2* might alter the transport activity for the drug and elevate the systemic circulation level of irinotecan, leading to severe myelosuppression.
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INTRODUCTION

Irinotecan, also known as CPT-11, is a semi-synthetic analog of camptothecin that is a natural alkaloid extract of plants such as *Camtotheca acuminate*.[1] As an anti-neoplastic agent of the class of topoisomerase 1 inhibitor, irinotecan has been widely used for the treatment of colorectal cancer, non-small-cell lung cancer and several other solid tumors.[2,3] However, the vast interindividual variability in the pharmacokinetics and pharmacodynamics of irinotecan and its metabolites rendered currently used irinotecan dosing strategy, which is solely based on the body surface area of patients, insufficient to perform personalized irinotecan therapy.[4,5] Consequently, the use of irinotecan is often limited by the unpredictable dose-limiting and life-threatening toxicities such as diarrhea[6] and myelosuppression that manifest as severe neutropenia, leukopenia, anemia and thrombocytopenia.[7]

Recent studies have implied not only that the increased systemic level of SN-38, the active metabolite of irinotecan, is correlated with irinotecan-induced severe myelosuppression[8] but also that the increased local level of SN-38 in the intestine was associated with life-threatening delayed-onset diarrhea.[9,10] Therefore, genetic polymorphisms in genes involved in metabolizing and transporting irinotecan, which may alter the pharmacokinetics of SN-38, have been extensively studied for their association with the interindividual variability in clinical outcome and toxicity of irinotecan-based therapy in recent years.[5,11,12]

The proteins that have critical roles in irinotecan metabolism include carboxylesterases (CES) and the two members of the cytochrome P450 family, CYP3A4 and CYP3A5. Although CES biotransforms irinotecan into SN-38 that is 100- to 1000-fold more potent than irinotecan,[13] CYP3A4 and CYP3A5 oxidize irinotecan to form two other metabolites, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin and 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin (NPC) that lack anticancer activity. However, it is now known that NPC can also be converted to SN-38 by CES.[14,15]

In addition, genes such as *UGT1A1*, *UGT1A7*, *UGT1A9*, *ABCC2*, *ABCG2* and *ABCB1* encode proteins that have important roles in the detoxification and hepatobiliary disposition of irinotecan.

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Glucuronidation of SN-38 to form the inactive SN-38G by uridine diphosphate glucuronosyltransferase isoforms, primarily by UGT1A1 in the liver, is the major pathway to detoxify irinotecan,[16] whereas the subsequent hepatobiliary excretion of irinotecan and its metabolites (SN-38 and SN-38G)[16,17] is mediated by transmembrane drug transporters such as ABCC2, ABCG2 and ABCB1.[5,12,18]

The prospective study conducted by Innocenti *et al.*[19] has established the association of the *UGT1A1*28* variant with severe toxicity and laid the foundation for genotype-based irinotecan dosing strategy. Many studies since then have revealed that other genetic polymorphisms in the *UGT1A1* gene or in other genes that are involved in the metabolism and disposition pathways of irinotecan also predisposed individual subjects to the risk of severe adverse drug reactions (ADRs).[11,20–23]

In this study, we genotyped and comprehensively analyzed 170 single nucleotide polymorphisms (SNPs) in 14 genes that possibly have essential roles in the metabolism and transport of irinotecan [24–26] to verify SNPs associated with severe myelosuppression in the Japanese population (Figure 1). We then fine-mapped the genomic region containing the associated SNP, performed haplotype analysis and elucidated its association with severe myelosuppression in the Japanese population.

MATERIALS AND METHODS

Subjects

This study was a retrospective case–control association study performed in two stages.[27] In both stages, the cases refer to patients who developed severe myelosuppression (such as leukopenia, neutropenia and/or anemia of grade 3 or 4) during irinotecan therapy, whereas controls refer to subjects who did not show any sign of ADR during irinotecan therapy. Grade of ADR was classified according to the National Cancer Institute—Common Toxicity Criteria version 2.0.[28]

The first stage involved 344 subjects who received irinotecan therapy and who were registered into 'the Leading Project for Personalized Medicine,' or the BioBank Japan Project, in the Ministry of Education, Culture, Sports, Science and Technology, Japan, from June 2003 to December 2007.[29] The BioBank Japan Project started in 2003 with the goal of collecting DNA and serum samples, along with clinical information, from 300 000 cases diagnosed with any of the 47 different diseases from a collaborative network of 66 hospitals in Japan. The biological materials and clinical information were collected from patients with written informed consent by medical coordinators at participating institutes.

Among the 344 aforementioned subjects, 23 and 58 subjects were each classified as cases and controls, whereas the remaining subjects developed either myelosuppression of lower grades or other ADRs such as nausea, vomiting, anorexia and/or diarrhea.

The second stage involved 63 subjects (39 subjects from Tokushukai Hospital Groups and 24 subjects from Sapporo Medical University) who received irinotecan therapy. Among these, 7 and 20 subjects were categorized as cases and controls, respectively. Table 1 describes the demographic characteristics of patients who participated in this study.

This project was approved by the ethics committees at The Institute of Medical Science, The University of Tokyo, Tokushukai Hospital Groups and Sapporo Medical University, Japan.

Selection of SNPs and design of the study

For each individual who participated in the first stage, a total of 170 SNPs (tagSNPs (tSNPs) or cSNPs) in or near to the 14 candidate genes involved in the metabolism and transporting pathways of irinotecan (*ABCB1*, *ABCC1*, *ABCC2*, *ABCC5*, *ABCG2*, *BCHE*, *CES2*, *CYP3A4*, *CYP3A5*, *SLCO1B1*, *SLCO1B3*, *UGT1A1*, *UGT1A7* and *UGT1A9*) were genotyped. As a whole, 27, 45, 12, 21, 8, 4, 9, 2, 1, 15, 18, 3, 4 and 1 SNPs were genotyped for each of the listed genes, respectively. All the tSNPs investigated in this study have reported minor allele frequencies of greater than 10% in the Japanese HapMap database (http://www.hapmap.org/) and capture most of the haplotypes in linkage disequilibrium (LD) blocks encompassing each gene ($r^2 > 0.8$). However, lacking information on tSNPs, only several functional SNPs were tested for *CYP3A4*, *CYP3A5*, *UGT1A1*, *UGT1A7* and *UGT1A9*. All SNPs were genotyped by using either the multiplex polymerase chain reaction-based Invader assay[30] or by direct sequencing as were described in Cha *et al.*[31]

Statistical analyses

The genotype and allele frequencies of each SNP were calculated and tested with the standard χ^2-test of the Hardy–Weinberg equilibrium (HWE).[32] SNPs that showed a deviation from the HWE ($P_{\chi 2} < 0.05$) in the control sample were eliminated from the subsequent analyses. Associations of each SNP with severe myelosuppression were evaluated by using Fisher's exact tests that consider each of the allelic, dominant-inheritance and recessive-inheritance models. SNPs showing a P_{Fisher} value of 0.01 or smaller in the first stage were considered as

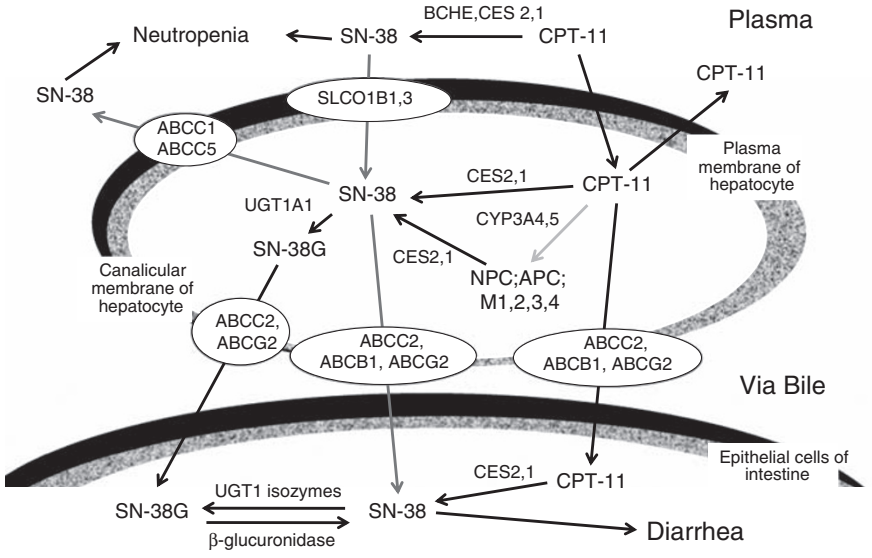

Figure 1 Disposition and metabolic pathways of irinotecan (CPT-11).

Table 1 Demographical characteristic of patients

	First study				*Second study*				*Combined study*			
	Case (N=*23*)		*Control* (N=*58*)		*Case* (N=*7*)		*Control* (N=*20*)		*Case* (N=*30*)		*Control* (N=*78*)	
Age	62.04 (37–77)		60.29 (34–77)		60.29 (40–81)		67.60 (50–84)		61.63 (37–81)		62.21 (34–84)	
% of men	61		71		57		42		60		65	
Types of cancers												
Lung	12	52%	10	17%	2	29%	4	17%	14	47%	14	18%
Cervical	6	26%	2	3%	0	0%	0	0%	6	20%	2	3%
Colorectal	3	13%	35	60%	3	43%	13	57%	6	20%	48	62%
Gastric	1	4%	4	7%	1	14%	2	9%	2	7%	6	8%
Ovarian	0	0%	2	3%	1	14%	1	4%	1	3%	3	4%
Breast+cervical	1	4%	0	0%	0	0%	0	0%	1	3%	0	0%
Pancreatic	0	0%	1	2%	0	0%	0	0%	0	0%	1	1%
Esophageal	0	0%	1	2%	0	0%	0	0%	0	0%	1	1%
Breast	0	0%	1	2%	0	0%	0	0%	0	0%	1	1%
No information	0	0%	2	0%	0	0%	0	0%	0	0%	2	3%
Types of regimens												
CPT-11	1	4%	11	19%	1	14%	2	9%	2	6%	13	17%
Other drug combinations	22	96%	47	81%	6	86%	18	91%	28	94%	65	83%

candidate SNPs that might be associated with severe myelosuppression and were further investigated in the second stage. As the number of subjects in the replication study is small, to increase the power of the study, genotyping results from both stages were combined and analyzed jointly. Bonferroni correction was applied for the judgment of statistical significance of the combined analysis.

Fine-mapping and haplotype analysis

According to the HapMap Japanese population database, other SNPs, which are in $r^2>0.3$ with the SNP showing a significant association with severe myelosuppression in this study, were further genotyped by the Invader assay. In addition, haplotype analysis for the fine-mapped region was also performed by Haploview software v4.1.[33]

RESULTS

Patient characteristics

A total of 108 patients were enrolled (81 in the first stage and 27 in the second stage) in this study. Nearly half of these subjects suffered from colorectal cancer (47 and 59%, respectively). The remaining patients suffered from lung cancer (27 and 22%, respectively), gastric cancer and ovarian cancer. Overall distributions of gender (male/female) were 18/12 in cases and 51/27 in controls (P=0.658). There was no distinctive difference in age distribution (median, years (range)) between the ADR and non-ADR groups (61.63 (37–81) versus 62.21 (34–84)). The patients with or without concomitant anticancer drugs were 2/28 or 13/65 in cases/controls, suggesting no significant difference (P=0.227). No significant difference was observed in the incidence of severe myelosuppression between patients of the first and second stages (23/58 versus 7/20) (P=1.00).

Association study of SNPs with irinotecan-induced severe myelosuppression

In the first stage, 23 patients who developed severe myelosuppression and 58 patients who did not show any signs of ADR after receiving irinotecan therapy were genotyped for a total of 170 SNPs in or near to the 14 genes that have critical roles in the metabolism and transport of irinotecan. We then examined associations of these SNPs with severe myelosuppression. Detailed information of the 170 SNPs and the results of association analyses with severe myelosuppression in the first stage of the study were summarized in Table 2.

In the process of the quality control of genotyping data, we excluded seven SNPs the genotype frequencies of which showed significant deviation from the HWE ($P_{\chi 2}<0.05$) in the control samples for further analyses. In addition, five markers that were found to be non-polymorphic in our samples of the first stage were excluded from subsequent analyses. Furthermore, we excluded eight SNPs (two in *UGT1A7*, two in *ABCB1*, one in *ABCC2*, two in *SLCO1B3* and one in *ABCC1*) that were absolutely linked to another SNP in our study (r^2=1, as were determined by Haploview 4.1 software[33]) from the subsequent analyses (Table 2).

Among the remaining 150 SNPs, ten showed minimum P_{Fisher} value of 0.05–0.01; these SNPs are located in the *ABCB1* (three SNPs), *ABCC1* (four SNPs), *UGT1A1* (one SNP) and *SLCO1B3* (two SNPs) (Table 2). In addition, five SNPs, two in the *SLCO1B3* and one in each of the *ABCC1*, *UGT1A7* and *ABCG2,* showed minimum P_{Fisher} value of less than 0.01. We considered these five SNPs as possible candidates and further genotyped them by using DNA samples of the 27 subjects who participated in the second stage of the study. These include 7 patients who developed severe myelosuppression and 20 who did not show any signs of ADR by the irinotecan therapy. Among the five SNPs examined, only one showed $P_{\mathrm{Fisher}}<0.05$ in the second stage of the study. This SNP, rs2622604, is located in intron 1 of the *ABCG2* gene (P_{Fisher}=0.0419) (Table 3).

Besides, when Bonferroni correction for multiple testing (based on 150 independent tests) was applied for judgment of statistical significance ($\alpha<0.000333$) in the combination analysis, the SNP rs2622604 was the only one showing a significant level of association with severe myelosuppression, with a Bonferroni-corrected P-value of smaller than 0.05 (P_{Fisher}=0.000237, $P_{\mathrm{Corrected}}$=0.036). In addition to this SNP, rs7977213 in *SLCO1B3* as well as the *UGT1A7*3* variant

Table 2 Associations of 170 SNPs with severe myelosuppression in subjects who received irinotecan therapy

			Allele		*Frequency of allele 1*		*Fisher test's* P-*values*			
Gene name	*SNP ID*	*Position of SNP/functional SNP*	*1*	*2*	[d]*Case*	[e]*Control*	[f]*1vs2*	[g]*11vs*	[h]*22vs*	*Odds ratio*
UGT1A9	rs3832043	UGT1A9*1b or *22	9T	10T	0.63	0.63	1.00E+00	8.05E−01	7.33E−01	0.76
UGT1A7	rs17868323	Exon 1 (Lys 129 Asn)	T	G	0.54	0.63	3.72E−01	1.00E+00	1.16E−01	2.68
UGT1A7	1A7_R131Ka[a]	Exon 1 (R131K) (UGT1A7*2)	C	A	0.54	0.63	3.72E−01	1.00E+00	1.16E−01	2.68
UGT1A7	1A7_R131Kb[a]	Exon 1 (R131K) (UGT1A7*2)	G	A	0.54	0.63	3.72E−01	1.00E+00	1.16E−01	2.68
UGT1A7	rs11692021	Exon 1 (Arg 208 Trp) (UGT1A7*3)	T	C	0.65	0.76	1.71E−01	1.00E+00	6.72E−03	15.56
UGT1A1	rs887829	Intron (tagged UGT1A1*28, promoter indel)	G	A	0.96	0.90	3.53E−01	1.64E−01	1.00E+00	5.15
UGT1A1	rs4148323	Exon 1 (Arg 71 Gly) (UGT1A1*6)	G	A	0.76	0.78	8.34E−01	4.51E−01	2.14E−02	12.00
UGT1A1	rs35350960	Exon 1 (Gln 229 Pro) (UGT1A1*27)	C	A	1.00	0.99	1.00E+00	1.00E+00	1.00E+00	NA
BCHE	rs697356	3′ near gene	G	C	0.30	0.40	2.74E−01	4.95E−01	4.46E−01	1.64
BCHE	rs1007845	3′ near gene	G	A	0.83	0.78	6.67E−01	6.13E−01	1.00E+00	1.40
BCHE	rs2048493	Intron 2	C	G	0.78	0.62	6.41E−02	5.08E−02	4.29E−01	2.74
BCHE	rs4639017	Intron 1	C	G	0.72	0.60	2.07E−01	2.07E−01	7.17E−01	2.07
ABCC5	rs6810123	3′ near gene	A	G	0.33	0.41	3.72E−01	1.00E+00	2.07E−01	2.07
ABCC5	rs12638772	3′ near gene	A	G	0.36	0.32	7.07E−01	2.50E−01	1.00E+00	2.36
ABCC5	rs7613247	3′ near gene	A	G	0.93	0.94	1.00E+00	1.00E+00	1.00E+00	1.15
ABCC5	rs2176825	3′ near gene	A	G	0.25	0.37	1.81E−01	5.07E−01	2.07E−01	1.98
ABCC5	rs13066518	3′ near gene	T	A	0.33	0.39	4.78E−01	1.00E+00	4.50E−01	1.62
ABCC5	rs3749443	3′ near gene	A	G	0.20	0.18	1.00E+00	1.00E+00	7.90E−01	1.29
ABCC5	rs1402001	3′ near gene	A	G	0.52	0.57	6.03E−01	7.95E−01	2.30E−01	2.10
ABCC5	rs6790814	3′ near gene	C	G	0.66	0.76	2.33E−01	4.63E−01	1.25E−01	4.42
ABCC5	rs9838667	3′ near gene	T	G	0.34	0.46	2.12E−01	5.64E−01	3.05E−01	1.85
ABCC5	rs2280392	3′ near gene	G	A	0.25	0.23	8.35E−01	3.40E−01	8.02E−01	2.84
ABCC5	rs1879257	3′ near gene	A	G	0.43	0.32	2.02E−01	3.07E−01	3.27E−01	2.02
ABCC5	rs3817403	3′ near gene	A	G	0.87	0.90	7.82E−01	1.00E+00	1.00E+00	1.19
ABCC5	rs3805111	3′ near gene	T	C	0.09	0.10	7.85E−01	1.00E+00	7.72E−01	1.24
ABCC5	rs3805108[b]	3′ near gene	A	G	0.11	0.19	2.50E−01	6.69E−01	4.00E−01	1.97
ABCC5	rs2872247	3′ near gene	T	G	0.76	0.70	4.49E−01	6.30E−01	6.67E−01	1.30
ABCC5	rs2293001	3′ near gene	T	C	0.48	0.54	6.00E−01	7.79E−01	5.56E−01	1.44
ABCC5	rs4148572	Intron 2	C	G	0.33	0.22	1.60E−01	1.36E−01	3.30E−01	4.20
ABCC5	rs4148568	Intron 2	A	G	0.13	0.18	6.37E−01	5.85E−01	7.89E−01	NA
ABCC5	rs4148564	Intron 2	A	G	0.89	0.82	3.44E−01	3.00E−01	1.00E+00	1.89
ABCC5	rs4148560	Intron 2	A	T	0.78	0.80	8.30E−01	7.96E−01	1.36E−01	4.20
ABCC5	rs7624838	Intron 2	T	C	0.50	0.52	8.63E−01	7.82E−01	1.00E+00	1.26
ABCG2	rs2231164	Intron 14	C	T	0.36	0.39	8.56E−01	5.57E−02	4.46E−01	NA
ABCG2	rs2622611	Intron 10	T	G	0.16	0.18	8.20E−01	3.29E−01	1.00E+00	NA
ABCG2	rs1871744	Intron 6	T	C	0.55	0.69	9.76E−02	3.22E−01	1.71E−01	2.73
ABCG2	rs2231142	Exon 5 (Gln 141 Lys)	C	A	0.78	0.78	1.00E+00	1.00E+00	1.00E+00	0.79
ABCG2	rs2231137	Exon 2 (Val 12 Met)	G	A	0.67	0.79	1.53E−01	3.19E−01	1.36E−01	4.20
ABCG2	rs1564481	Intron 1	C	T	0.72	0.62	2.77E−01	3.15E−01	6.67E−01	1.74
ABCG2	rs2622624	Intron 1	A	G	0.41	0.29	1.93E−01	2.78E−01	3.35E−01	2.41
ABCG2	rs2622604	Intron 1	T	C	0.28	0.09	2.35E−03	7.81E−02	6.66E−03	4.40
ABCB1	rs1882478	Intron 27	C	T	0.59	0.55	7.27E−01	4.31E−01	7.57E−01	1.57
ABCB1	rs6979885	Intron 27	G	A	0.89	0.91	1.00E+00	1.00E+00	1.00E+00	1.19
ABCB1	rs2235047	Intron 27	T	G	0.50	0.52	8.63E−01	7.82E−01	1.00E+00	1.26
ABCB1	rs1045642	Exon 27 (Ile 3 Ile)	T	C	0.37	0.44	4.80E−01	1.00E+00	4.34E−01	1.67
ABCB1	rs1002205	Intron 26	C	G	0.52	0.60	3.75E−01	4.21E−01	7.14E−01	1.70
ABCB1	rs6949448	Intron 26	T	C	0.35	0.40	5.91E−01	1.00E+00	6.09E−01	1.42
ABCB1	rs2235067	Intron 23	A	G	0.07	0.11	5.60E−01	1.00E+00	5.36E−01	1.83
ABCB1	rs2373588	Intron 22	T	C	0.43	0.39	5.98E−01	7.20E−01	7.99E−01	1.53
ABCB1	rs7787082	Intron 22	A	G	0.50	0.50	1.00E+00	7.72E−01	7.72E−01	NA
ABCB1	rs3789246	Intron 20	A	G	0.09	0.19	1.50E−01	5.51E−01	2.69E−01	2.31
ABCB1	rs1922242[b]	Intron 17	A	T	0.78	0.68	2.45E−01	3.24E−01	4.90E−01	1.74
ABCB1	rs2235046	Intron 17	A	G	0.72	0.59	1.51E−01	4.38E−02	1.00E+00	2.89
ABCB1	rs868755	Intron 9	C	A	0.59	0.61	1.00E+00	1.00E+00	7.29E−01	1.36
ABCB1	rs4148734	Intron 8	C	T	0.87	0.90	7.79E−01	5.42E−01	1.00E+00	1.55
ABCB1	rs2235018	Intron 6	A	G	0.67	0.81	9.65E−02	2.09E−01	1.40E−01	4.13
ABCB1	rs10256836[a]	Intron 5	C	G	0.13	0.09	5.68E−01	1.00E+00	5.31E−01	1.73
ABCB1	rs10259849[a]	Intron 5	C	T	0.14	0.10	5.75E−01	1.00E+00	5.36E−01	1.65
ABCB1	rs1202172	Intron 5	T	G	0.96	0.86	1.01E−01	8.01E−02	1.00E+00	4.00

Table 2 Continued

			Allele		Frequency of allele 1		Fisher test's P-values			
Gene name	*SNP ID*	*Position of SNP/functional SNP*	*1*	*2*	[d]*Case*	[e]*Control*	[f]*1vs2*	[g]*11vs*	[h]*22vs*	*Odds ratio*
ABCB1	rs17327442	Intron 5	T	A	0.89	0.94	5.12E−01	4.93E−01	1.00E+00	1.87
ABCB1	rs1202184	Intron 5	G	A	0.24	0.33	3.44E−01	1.00E+00	2.25E−01	1.91
ABCB1	rs3789243	Intron 4	T	C	0.50	0.31	2.81E−02	5.05E−02	1.38E−01	3.50
ABCB1	rs3213619	Exon 2 (5′ UTR)	C	T	0.02	0.10	1.81E−01	1.00E+00	1.63E−01	5.02
ABCB1	rs4148732	Intron 1	A	G	0.87	0.96	7.89E−02	6.87E−02	1.00E+00	3.67
ABCB1	rs13233308	Intron 1	T	C	0.43	0.44	1.00E+00	7.60E−01	7.95E−01	0.82
ABCB1	rs1978095	Intron 1	T	C	0.67	0.73	5.59E−01	6.21E−01	6.89E−01	1.36
ABCB1	rs2157929	Intron 1	T	C	0.93	0.79	3.32E−02	5.60E−02	5.51E−01	3.81
ABCB1	rs10278483	Intron 1	T	C	0.96	0.86	1.01E−01	1.36E−01	5.88E−01	3.34
CYP3A5	rs776746	Intron 3 (CYP3A5*3)	A	G	0.22	0.32	2.50E−01	6.67E−01	3.26E−01	1.79
CYP3A4	rs28371759[c]	Exon 10 (Pro 293 Leu) (CYP3A4*18)			1.00	1.00	1.00E+00	1.00E+00	1.00E+00	NA
CYP3A4	rs12721627[b]	Exon 7 (Ser 185 Thr) (CYP3A4*16)	C	G	1.00	0.96	3.23E−01	3.22E−01	1.00E+00	NA
ABCC2	rs12268782	5′ near gene	A	G	0.22	0.13	2.26E−01	1.00E+00	1.81E−01	2.21
ABCC2	rs2804398	Intron 7	T	A	0.83	0.84	8.14E−01	7.90E−01	1.00E+00	1.29
ABCC2	rs2756109	Intron 7	G	T	0.63	0.65	8.55E−01	1.00E+00	1.00E+00	1.12
ABCC2	rs2273697	Exon 10 (Ile 417 Val)	G	A	0.83	0.92	8.91E−02	5.96E−02	1.00E+00	3.33
ABCC2	rs11190291[a]	Intron 11	T	C	0.17	0.08	8.91E−02	1.00E+00	5.96E−02	3.33
ABCC2	rs2002042	Intron 19	T	C	0.33	0.39	4.78E−01	2.78E−01	1.00E+00	3.52
ABCC2	rs17222723[c]	Exon 25 (Glu 1188 Val)	T	A	1.00	1.00	1.00E+00	1.00E+00	1.00E+00	NA
ABCC2	rs3740065[b]	Intron 29	T	C	0.65	0.60	5.96E−01	6.18E−01	9.90E−02	6.36
ABCC2	rs12762549	3′ near gene	C	G	0.48	0.36	2.13E−01	1.00E+00	6.81E−02	3.35
ABCC2	rs2862691	3′ near gene	T	C	0.19	0.25	5.26E−01	6.05E−01	6.11E−01	NA
ABCC2	rs11598781	3′ near gene	T	C	0.17	0.27	2.30E−01	1.00E+00	1.40E−01	2.29
ABCC2	rs11190303	3′ near gene	T	C	0.24	0.33	2.62E−01	5.33E−02	1.00E+00	NA
SLCO1B3	rs12228798	Intron 1	C	T	0.82	0.83	1.00E+00	7.77E−01	2.27E−01	NA
SLCO1B3	rs7977213	Intron 2	G	C	0.43	0.18	1.29E−03	1.31E−03	2.55E−02	NA
SLCO1B3	rs10841661	Intron 2	T	C	0.48	0.23	2.73E−03	5.65E−03	4.79E−02	9.88
SLCO1B3	rs4149118	Intron 4	A	G	0.59	0.48	3.31E−01	3.49E−01	5.40E−01	1.85
SLCO1B3	rs3764009[a]	Intron 4	A	G	0.68	0.69	1.00E+00	1.00E+00	1.00E+00	1.16
SLCO1B3	rs7311358	Exon 7 (Ile 233 Met)	A	G	0.68	0.69	1.00E+00	1.00E+00	1.00E+00	1.16
SLCO1B3	rs11045585[b]	Intron 12	G	A	0.13	0.21	2.76E−01	3.14E−01	6.06E−01	NA
SLCO1B3	rs980084	Intron 12	G	C	0.28	0.45	7.48E−02	3.80E−01	7.66E−02	2.67
SLCO1B3	rs3764006	Exon 14 (Gly 611 Gly)	T	C	0.87	0.70	2.74E−02	1.32E−01	9.75E−02	NA
SLCO1B3	rs919840	3′ near gene	C	G	0.97	0.90	2.99E−01	2.77E−01	1.00E+00	3.74
SLCO1B3	rs2117032	3′ near gene	T	C	0.52	0.47	6.05E−01	7.68E−01	7.82E−01	1.35
SLCO1B3	rs7312051	3′ near gene	C	T	1.00	0.91	1.26E−01	1.04E−01	1.00E+00	NA
SLCO1B3	rs10841714	3′ near gene	C	T	0.15	0.17	8.01E−01	1.00E+00	1.00E+00	NA
SLCO1B3	rs2174012	3′ near gene	T	C	0.62	0.63	1.00E+00	5.72E−01	4.55E−01	0.39
SLCO1B3	rs11045639[a]	3′ near gene	G	A	0.65	0.72	4.46E−01	2.17E−01	7.25E−01	2.05
SLCO1B3	rs2900459	3′ near gene	G	A	0.46	0.56	2.95E−01	1.43E−02	5.70E−01	5.96
SLCO1B3	rs4762693	3′ near gene	G	A	0.65	0.72	4.46E−01	2.17E−01	7.25E−01	2.05
SLCO1B3	rs10734711	3′ near gene	A	G	0.28	0.35	4.61E−01	2.78E−01	8.05E−01	3.52
SLCO1B1	rs12228427	5′ near gene (LST-3TM12 Intron 9)	A	G	0.96	0.88	1.59E−01	1.36E−01	1.00E+00	3.34
SLCO1B1	rs1910163	5′ near gene (LST-3TM12 Intron 12)	A	T	0.28	0.34	4.66E−01	7.25E−01	6.21E−01	1.44
SLCO1B1	rs6487207	5′ near gene (LST-3TM12 Intron 11)	T	G	0.78	0.73	5.54E−01	8.11E−01	3.22E−01	NA
SLCO1B1	rs1604539	5′ near gene (LST-3TM12 Intron 12)	T	G	0.80	0.75	5.41E−01	6.18E−01	1.00E+00	1.42
SLCO1B1	rs7973691	5′ near gene	T	C	0.80	0.83	8.21E−01	7.98E−01	1.00E+00	1.22
SLCO1B1	rs10743408	Intron 2	C	G	0.24	0.18	4.97E−01	1.00E+00	3.06E−01	1.73
SLCO1B1	rs976754	Intron 2	T	C	0.83	0.69	1.15E−01	8.67E−02	6.67E−01	2.54
SLCO1B1	rs2291073	Intron 3	T	G	0.78	0.72	4.35E−01	8.11E−01	1.76E−01	NA
SLCO1B1	rs4149037	Intron 4	A	G	0.70	0.80	2.12E−01	2.07E−01	6.19E−01	2.07
SLCO1B1	rs4149056	Exon 5 (Ala 174 Val)	T	C	0.72	0.84	8.02E−02	2.04E−01	8.01E−02	NA
SLCO1B1	rs2417967	Intron 11	A	G	0.33	0.21	1.54E−01	1.00E+00	8.68E−02	2.41
SLCO1B1	rs7969341[b]	Intron 14	T	C	0.50	0.46	7.24E−01	1.58E−01	5.60E−02	3.80
SLCO1B1	rs4149085	3′ UTR	T	C	0.74	0.66	4.53E−01	8.05E−01	1.76E−01	NA
SLCO1B1	rs12372067	3′ near gene	C	A	0.21	0.33	2.06E−01	1.00E+00	1.68E−01	2.42
SLCO1B1	rs12310063	3′ near gene	A	C	0.67	0.76	3.21E−01	2.13E−01	1.00E+00	2.07
ABCC1	rs8050881	5′ near gene	A	G	0.33	0.23	2.37E−01	1.00E+00	1.44E−01	2.13
ABCC1	rs4148330	5′ near gene	G	A	0.48	0.34	1.46E−01	2.95E−01	2.13E−01	2.02

Table 2 Continued

Gene name	SNP ID	Position of SNP/functional SNP	Allele 1	Allele 2	Frequency of allele 1 [d]Case	Frequency of allele 1 [e]Control	Fisher test's P-values [f]1vs2	[g]11vs	[h]22vs	Odds ratio
ABCC1	rs7190484	Intron 1	T	C	0.43	0.34	3.67E−01	5.03E−01	4.62E−01	1.52
ABCC1	rs215098	Intron 1	C	A	0.41	0.34	4.63E−01	1.96E−01	1.00E+00	2.30
ABCC1	rs215096	Intron 1	T	C	0.89	0.87	1.00E+00	5.63E−01	2.89E−01	0.00
ABCC1	rs152023	Intron 1	G	A	0.48	0.41	4.75E−01	3.79E−01	8.05E−01	1.79
ABCC1	rs6498595	Intron 1	G	C	0.41	0.31	2.70E−01	2.73E−02	1.00E+00	4.76
ABCC1	rs7196970	Intron 1	C	G	0.70	0.63	4.71E−01	3.25E−01	1.00E+00	1.73
ABCC1	rs12935283	Intron 1	A	G	0.67	0.61	4.78E−01	3.19E−01	1.00E+00	1.79
ABCC1	rs246240	Intron 5	A	G	0.60	0.58	1.00E+00	1.00E+00	1.00E+00	1.06
ABCC1	rs924138[b]	Intron 5	T	C	0.68	0.63	5.84E−01	6.17E−01	1.00E+00	1.38
ABCC1	rs2062541	Intron 6	C	T	0.71	0.64	4.46E−01	4.45E−01	7.51E−01	1.60
ABCC1	rs11647513	Intron 6	C	T	0.78	0.70	3.34E−01	2.14E−01	1.00E+00	2.13
ABCC1	rs35593	Intron 11	T	C	0.71	0.75	8.37E−01	1.00E+00	1.00E+00	2.70
ABCC1	rs3765129	Intron 11	C	T	0.98	0.84	2.69E−02	1.75E−02	1.00E+00	9.90
ABCC1	rs17287570	Intron 12	A	C	0.73	0.91	6.32E−03	1.03E−02	2.75E−01	4.27
ABCC1	rs35597	Intron 12	A	G	0.59	0.55	7.19E−01	5.85E−01	1.00E+00	1.45
ABCC1	rs35598[a]	Intron 12	A	G	0.82	0.86	6.21E−01	7.86E−01	2.78E−01	NA
ABCC1	rs9932506	Intron 12	G	A	0.72	0.75	6.94E−01	6.21E−01	1.00E+00	1.44
ABCC1	rs35604	Intron 12	G	A	0.24	0.25	1.00E+00	1.00E+00	1.00E+00	1.63
ABCC1	rs35606	Intron 13	C	T	0.82	0.86	6.17E−01	7.82E−01	2.86E−01	NA
ABCC1	rs35620	Intron 14	G	C	0.26	0.23	8.39E−01	1.00E+00	6.19E−01	1.39
ABCC1	rs35625	Intron 14	C	T	0.41	0.34	4.70E−01	7.52E−01	4.72E−01	1.45
ABCC1	rs35626	Intron 15	T	G	0.43	0.40	7.21E−01	5.08E−01	2.96E−01	1.93
ABCC1	rs4148353	Intron 15	T	G	0.20	0.07	2.42E−02	1.00E+00	1.69E−02	4.02
ABCC1	rs35629	Intron 15	C	T	0.80	0.76	5.46E−01	6.24E−01	1.00E+00	1.32
ABCC1	rs2269800	Intron 20	A	G	0.74	0.66	4.53E−01	8.06E−01	2.78E−01	3.52
ABCC1	rs11864374	Intron 21	G	A	0.78	0.77	8.41E−01	1.00E+00	1.00E+00	1.63
ABCC1	rs3887893	Intron 22	A	G	0.48	0.46	8.62E−01	2.30E−01	5.85E−01	2.10
ABCC1	rs4148376	Intron 23	A	G	0.93	0.95	1.00E+00	1.00E+00	1.00E+00	1.30
ABCC1	rs2238475	Intron 23	C	A	0.11	0.18	3.45E−01	1.00E+00	4.21E−01	1.80
ABCC1	rs212079	Intron 26	G	A	0.76	0.83	3.77E−01	1.00E+00	6.71E−02	8.55
ABCC1	rs2283512	Intron 26	G	T	0.48	0.46	1.00E+00	7.68E−01	1.00E+00	1.32
ABCC1	rs212081	Intron 27	T	C	0.20	0.22	8.34E−01	5.73E−01	4.61E−01	1.50
ABCC1	rs212084	Intron 28	G	A	0.68	0.77	2.90E−01	3.02E−01	6.00E−01	1.81
ABCC1	rs212087	Intron 28	C	T	0.57	0.70	1.41E−01	1.38E−01	3.45E−01	2.30
ABCC1	rs4148380	3′ UTR	G	A	0.87	0.89	7.88E−01	7.75E−01	1.00E+00	1.22
ABCC1	rs212091	3′ UTR	G	A	0.22	0.34	1.84E−01	2.68E−01	3.35E−01	4.04
ABCC1	rs12448760	3′ near gene	G	A	0.82	0.89	2.95E−01	3.79E−01	1.00E+00	1.79
ABCC1	rs9932935	3′ near gene (ABCC6 Intron 29)	A	T	0.96	0.91	5.12E−01	5.00E−01	1.00E+00	1.93
ABCC1	rs2066738	3′ near gene (ABCC6 Intron 28)	C	T	0.74	0.90	1.50E−02	5.28E−02	7.81E−02	2.95
ABCC1	rs169845	3′ near gene (ABCC6 Intron 27)	G	C	0.26	0.40	1.04E−01	3.28E−01	2.13E−01	2.07
ABCC1	rs2238471	3′ near gene (ABCC6 Intron 26)	A	C	0.93	0.81	5.49E−02	5.79E−02	1.00E+00	3.78
ABCC1	rs3213471	3′ near gene (ABCC6 Intron 24)	A	G	0.91	0.92	1.00E+00	7.33E−01	1.00E+00	1.32
ABCC1	rs3213473	3′ near gene (ABCC6 Intron 24)	T	G	0.16	0.13	7.98E−01	1.00E+00	7.71E−01	1.27
CES2	rs3843712	5′ near gene	G	A	0.18	0.12	4.41E−01	1.00E+00	4.00E−01	1.80
CES2	rs8062110	5′ near gene	G	C	0.61	0.65	7.16E−01	1.00E+00	7.28E−01	1.39
CES2	rs4783744	5′ near gene	G	A	0.66	0.62	8.37E−01	7.76E−01	1.00E+00	1.24
CES2	rs7194513	5′ near gene	G	A	0.26	0.27	1.00E+00	3.14E−01	6.29E−01	NA
CES2	rs28382812[c]	Exon 1 (Ile 37 Ile)	C	T	1.00	1.00	1.00E+00	1.00E+00	1.00E+00	NA
CES2	rs2241410	Intron 2	T	G	0.10	0.11	7.85E−01	1.00E+00	7.71E−01	1.26
CES2	rs2303218	Intron 2	G	A	0.09	0.21	1.03E−01	5.55E−01	1.15E−01	2.70
CES2	rs8192924[c]	Exon 5 (His 270 Arg)	G	A	1.00	1.00	1.00E+00	1.00E+00	1.00E+00	NA
CES2	rs28382827[c]	Exon 12 (Leu 613 Leu)	C	T	1.00	1.00	1.00E+00	1.00E+00	1.00E+00	NA

Abbreviations: Chrom, chromosome; HWE, Hardy–Weinberg equilibrium; NA, not available; SNP, single nucleotide polymorphism.
[a]SNPs absolutely linked with other SNP in our study
[b]SNPs with HWE test's *P*-value <0.05 in control group.
[c]Non-polymorphic markers in our study.
[d]Case: *N*=23.
[e]Control: *N*=58.
[f]1vs2: Allelic model of Fisher's exact test.
[g]11vs: Dominant or recessive-inheritance model of Fisher's exact test, depending on inheritance mode of allele 1.
[h]22vs: Dominant or recessive-inheritance model of Fisher's exact test, depending on inheritance mode of allele 2.

Table 3 Results of second and combined studies for SNPs associated with severe myelosuppression with Fisher test's $P<0.01$ in the first study

			Case			*Control*			*Fisher test's* P-*values*			
Gene	*SNP*	*Study*	*p(11)*	*p(12)*	*p(22)*	*p(11)*	*p(12)*	*p(22)*	[a]*1vs2*	[b]*11vs*	[c]*22vs*	*Odds ratio*
ABCC1	rs17287570	First	11	10	1	47	11	0	6.32E−03	1.03E−02	2.75E−01	4.27
		Second	5	2	0	14	6	0	1.00E+00	1.00E+00	1.00E+00	NA
		Combined	16	12	1	61	17	0	1.78E−02	2.83E−02	2.71E−01	2.92
ABCG2	rs2622604	First	2	9	12	0	10	48	2.35E−03	7.81E−02	6.66E−03	4.40
		Second	0	3	4	0	1	19	4.92E−02	1.00E+00	4.19E−02	14.25
		Combined	2	12	16	0	11	67	2.37E−04	7.53E−02	6.26E−04	5.33
SLCO1B3	rs10841661	First	6	10	7	2	23	33	2.73E−03	5.65E−03	4.79E−02	9.88
		Second	0	4	3	2	7	11	1.00E+00	6.01E−01	6.78E−01	0.00
		Combined	6	14	10	4	30	44	7.89E−03	2.64E−02	5.22E−02	4.63
SLCO1B3	rs7977213	First	5	10	8	0	21	37	1.29E−03	1.31E−03	2.55E−02	NA
		Second	0	3	4	0	7	13	1.00E+00	1.00E+00	1.00E+00	NA
		Combined	5	13	12	0	28	50	2.32E−03	1.28E−03	3.02E−02	NA
*UGT1A7*3*	rs11692021	First	12	6	5	31	25	1	1.71E−01	1.00E+00	6.72E−03	15.56
		Second	3	3	1	13	6	1	2.85E−01	3.91E−01	1.00E+00	2.48
		Combined	15	9	6	44	31	2	8.32E−02	5.24E−01	5.83E−03	9.38

[a]1vs2: Allelic model of Fisher's exact test.
[b]11vs: Dominant or recessive-inheritance model of Fisher's exact test, depending on inheritance mode of allele 1.
[c]22vs: Dominant or recessive-inheritance model of Fisher's exact test, depending on inheritance mode of allele 2.

Table 4 Fine-mapping of the *ABCG2* gene

		Case				*Control*				*Fisher test's* P-*values*				
SNP	*Study*	*p(11)*	*p(12)*	*p(22)*	*Sum*	*p(11)*	*p(12)*	*p(22)*	*Sum*	[a]*1vs2*	[b]*11vs*	[c]*22vs*	*Minimum*	*Odds ratio*
rs12641369	First	3	10	9	22	2	19	36	57	4.10E−02	1.29E−01	8.30E−02	4.10E−02	2.48
rs12641369	Second	1	3	3	7	0	7	13	20	2.61E−01	2.59E−01	3.91E−01	2.59E−01	NA
rs12641369	Combined	4	13	12	29	2	26	49	77	1.28E−02	4.64E−02	4.84E−02	1.28E−02	6.00
rs4148152	First	3	9	10	22	2	19	36	57	9.54E−02	1.29E−01	2.04E−01	9.54E−02	4.34
rs4148152	Second	1	3	3	7	0	7	13	20	2.61E−01	2.59E−01	3.91E−01	2.59E−01	NA
rs4148152	Combined	4	12	13	29	2	26	49	77	2.91E−02	4.64E−02	1.21E−01	2.91E−02	6.00
rs2231137	First	10	9	3	22	36	19	2	57	9.54E−02	2.04E−01	1.29E−01	9.54E−02	4.34
rs2231137	Second	3	3	1	7	13	7	0	20	2.61E−01	3.91E−01	2.59E−01	2.59E−01	NA
rs2231137	Combined	13	12	4	29	49	26	2	77	2.91E−02	1.21E−01	4.64E−02	2.91E−02	6.00
rs4148150	First	3	9	11	23	2	16	36	54	6.22E−02	1.54E−01	1.34E−01	6.22E−02	2.18
rs4148150	Second	1	0	3	4	0	3	13	16	5.63E−01	2.00E−01	1.00E+00	2.00E−01	NA
rs4148150	Combined	4	9	14	27	2	19	49	70	2.86E−02	4.89E−02	1.03E−01	2.86E−02	5.91
rs3109823	First	9	11	3	23	47	10	0	57	4.81E−05	2.47E−04	2.16E−02	4.81E−05	7.31
rs3109823	Second	5	2	0	7	19	1	0	20	1.61E−01	1.56E−01	1.00E+00	1.56E−01	7.60
rs3109823	Combined	14	13	3	30	66	11	0	77	1.33E−05	7.11E−05	2.05E−02	1.33E−05	6.86
rs2622604	First	2	9	12	23	0	9	48	57	1.35E−03	8.01E−02	4.51E−03	1.35E−03	4.89
rs2622604	Second	0	3	4	7	0	1	19	20	4.92E−02	1.00E+00	4.19E−02	4.19E−02	14.25
rs2622604	Combined	2	12	16	30	0	10	67	77	1.40E−04	7.67E−02	3.75E−04	1.40E−04	5.86
rs2622605	First	5	9	9	23	5	20	32	57	8.73E−02	1.41E−01	2.19E−01	8.73E−02	2.89
rs2622605	Second	0	5	2	7	1	5	14	20	2.61E−01	1.00E+00	8.40E−02	8.40E−02	5.83
rs2622605	Combined	5	14	11	30	6	25	46	77	2.79E−02	2.85E−01	5.12E−02	2.79E−02	2.56

[a]1vs2: Allelic model of Fisher's exact test.
[b]11vs: Dominant or recessive-inheritance model of Fisher's exact test, depending on inheritance mode of allele 1.
[c]22vs: Dominant or recessive-inheritance model of Fisher's exact test, depending on inheritance mode of allele 2.

revealed a relatively small *P*-value in the combination analysis, although it did not reach a significant level after Bonferroni correction.

Fine mapping of the *ABCG2* gene and haplotype analysis

We further genotyped six other SNPs in the *ABCG2* gene that, according to the HapMap database of the Japanese population, are

Table 5a Frequencies of associated allele and corresponding *P*-values for SNPs examined for *ABCG2*

SNP ID	Associated allele	Case, control frequencies	χ^2-test's P-value
rs12641369	A	0.362, 0.195	1.11E−02
rs4148152	C	0.345, 0.195	2.18E−02
rs2231137	T	0.345, 0.195	2.18E−02
rs4148150	T	0.315, 0.164	2.02E−02
rs3109823	C	0.317, 0.071	3.46E−06
rs2622604	T	0.267, 0.065	4.96E−05
rs2622605	T	0.400, 0.240	2.01E−02

Table 5b Frequencies of haplotypes and corresponding *P*-values for haplotypes of *ABCG2*

Block	Haplotype frequencies	Case, control frequencies	χ^2-test's P-value
Block 1			
GTCC	0.754	0.638, 0.798	1.53E−02
ACTT	0.224	0.342, 0.180	1.15E−02
ACTC	0.012	0.003, 0.015	4.53E−01
Block 2			
TC	0.850	0.666, 0.922	2.49E−06
CT	0.112	0.250, 0.058	6.71E−05
CC	0.028	0.067, 0.013	3.24E−02

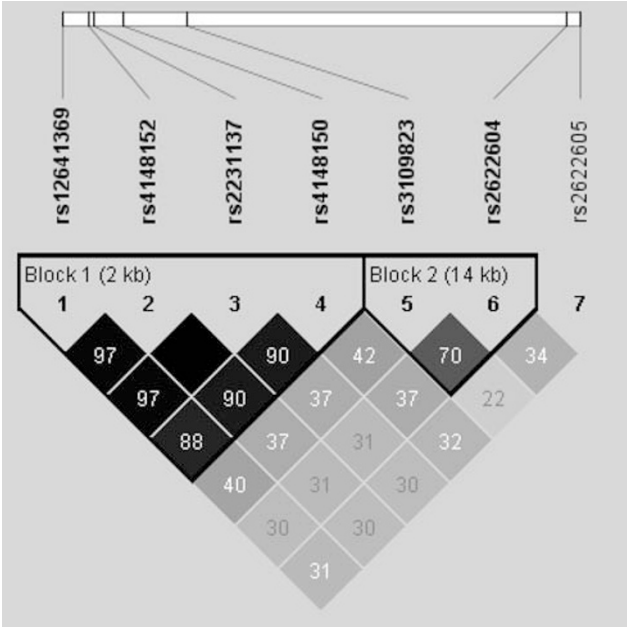

Figure 2 Haplotype structure of the *ABCG2* gene. Pairwise LD is displayed in black (strong LD) and gray (low LD) rectangles. Values represent r^2 measurements.

in $r^2 > 0.3$ with rs2622604. We identified another SNP, rs3109823, which shows a stronger association with severe myelosuppression (P_{Fisher}=0.0000133; $P_{\chi 2}$=3.46E-06) (Tables 4 and 5a). Haplotype analysis that considered the seven SNPs of the *ABCG2* gene revealed that two haplotypes, consisting of the two aforementioned SNPs, showed an association as strong as the independent effects of the two SNPs (haplotype TC, $P_{\chi 2}$=2.49E-06; haplotype CT, $P_{\chi 2}$=6.71E-05) (Table 5b) (Figure 2).

DISCUSSION

This study is a case–control association study in a retrospective design. Although the pharmacokinetic data for each subject could not be obtained, correlations between severe myelosuppression with 170 loci had been directly examined. In addition, statistical support that an intronic SNP of the *ABCG2* gene, rs2622604, was likely to be associated with irinotecan-induced severe myelosuppression (grade 3 or 4) has been shown. On top of that, we have also fine-mapped the *ABCG2* gene and identified another SNP, rs3109823, which showed stronger association with severe myelosuppression.

ABCG2 (ATP-binding cassette, Subfamily G, Member 2) encodes a transmembrane protein that mediates the hepatobiliary excretion of SN-38 and may have a major role in the pharmacokinetics of irinotecan.[34] In addition to that, several studies have also reported that the overexpression of ABCG2 is associated with a decrease in the intracellular concentration of SN-38, leading to making cancer cells SN-38/irinotecan-resistant.[35–38] In this study, two SNPs in *ABCG2* have been associated with severe myelosuppression that might be associated with an elevated level of SN-38 in systemic circulation. Furthermore, haplotypes containing the two SNPs have also shown a similarly strong association with severe myelosuppression. Although downstream functional analyses and pharmacokinetic studies were not performed to provide additional supporting evidences on the functional relevance of the two SNPs for severe myelosuppression and we have not pinpointed the causative SNP, a recent study by Poonkuzhali *et al.*[39] has provided evidence that the SNP, rs2622604, was associated with a lower mRNA expression of *ABCG2*. Their results support our hypothesis that individuals who carry the risk genotypes for rs2622604 might have reduced hepatobiliary efflux activity of SN-38, leading to elevated intracellular concentration of SN-38 in hepatocytes. This subsequently causes the accumulation of irinotecan/SN-38 in the systemic circulation and induces severe myelosuppression. On the other hand, although rs3109823 revealed stronger association with severe myelosuppression in our study, this SNP did not seem to be associated with reduced mRNA expression according to the study of Poonkuzhali *et al.*[39] The strong association of this SNP with severe myelosuppression might simply be because of its strong LD with rs2622604 (r^2=0.70). Further functional analysis on these SNPs in the *ABCG2* gene may provide additional evidence to support our hypothesis.

In addition to the *ABCG2* gene, we also investigated associations of *UGT1A* variants such as *UGT1A1*28*, *UGT1A1*6*, *UGT1A1*27* and *UGT1A7*3* with severe myelosuppression in the exploratory study. We did not observe strong associations of *UGT1A1*28* and *UGT1A1*27* variants with severe myelosuppression possibly because of the low allelic frequencies of these variants in the subjects we examined. Although the *UGT1A1*6* variant, which is more prevalent in the Asian population, revealed a weak association (P_{Fisher}=0.0214) (Table 2) with severe myelosuppression, we did not further investigate this variant because we observed much stronger association of the *UGT1A7*3* variant than the *UGT1A1*6* variant. In our study, rs11692021 that represents the **3* variant of the UGT1A7, which was reported to have the highest glucuronidation rate of SN-38 among UGT1A isoforms *in vitro*,[40,41] showed the strongest association with severe myelosuppression (P_{Fisher}=0.00583). Although none

of the *UGT1A* variants showed significant association with severe myelosuppression in our study, the tendency of association could be observed. Thus, examining the association of these variants with severe myelosuppression in a larger number of subjects should be rewarding. As these variants are in strong LD,[42] we need to further analyze which *UGT1A* variant(s) has biological significance.

Although the number of subjects investigated in this study is limited and the associations of several variants, such as those in the *UGT1A* genes, need to be further validated by examining a larger number of subjects, we have successfully identified an intronic SNP in the *ABCG2* gene to be significantly associated with irinotecan-induced severe myelosuppression. Our results suggest that genetic polymorphism in *ABCG2* might alter transport activity for the drug and elevate the level of irinotecan in systemic circulation, leading to severe myelosuppression.

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