GWAS of 972 autologous stem cell recipients with multiple myeloma identifies 11 genetic variants associated with chemotherapy-induced oral mucositis

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Abstract

Purpose High-dose chemotherapy and autologous stem cell transplant (ASCT) to treat multiple myeloma (MM) and other cancers carries the risk of oral mucositis (OM) with sequelae including impaired nutritional and fluid intake, pain, and infectious complications. As a result of these problems, cancer treatment may have to be interrupted or delayed. In this study, we looked beyond OM's known risk factors of renal function and melphalan dose with a genome-wide association study (GWAS) to evaluate whether genetic variants in conjunction with clinical risk factors influence predisposition for OM.

Methods Genotyping was performed using Illumina HumanOmni1-Quad v1.0 BeadChip and further assessed for data quality. We tested 892,589 germline single-nucleotide polymorphisms (SNPs) for association with OM among 972 Caucasian patients treated with high-dose melphalan and ASCT in

Total Therapy clinical trials (TT2, TT3, TT4) for newly diagnosed MM. Statistical analyses included *t* tests, stepwise regression modeling, and logistic regression modeling to find baseline clinical factors and genotypes associated with OM.

Results We found that 353 (36.3 %) patients had grades 2–4 OM. Type of treatment protocol, baseline estimated glomerular filtration rate, and melphalan dose along with baseline serum albumin and female gender predicted 43.6 % of grades 2–4 OM cases. Eleven SNPs located in or near matrix metalloproteinase 13, JPH3, DHRS7C, CEP192, CPEB1/LINC00692, FBN2, ALDH1A1, and DMRTA1/FLJ35282 were associated with grades 2–4 OM. The addition of these SNPs increased sensitivity in detecting grades 2–4 OM cases to 52 %.

Conclusions These SNPs may be important for their roles in inflammatory pathways, epithelial healing, and chemotherapy detoxification.

This work was performed at the University of Arkansas for Medical Sciences, Little Rock, AR.

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High-dose chemotherapy with autologous stem cell transplantation (HD-ASCT) provides a survival benefit for patients with various hematologic cancers, particularly those with multiple myeloma receiving melphalan-based conditioning regimens [1]. Although higher melphalan doses are associated with increased remission rates and event-free and overall survival compared to lower melphalan doses [1, 2], the substantial toxicity burden of this regimen, particularly mucosal damage, may limit feasible use. Oral mucositis (OM), the dose-limiting toxicity of high-dose melphalan (HD-MEL), is common, frequently severe [3], and associated with various complications including pain, malnutrition, severe infections, and even death [4–7]. Treatment for these complications increases the costs of care [4, 8], and severe OM may lead to chemotherapy dose reduction or discontinuation that jeopardizes patient outcomes [8]. An effective mucoprotective agent [9] recently became available, again increasing the cost of care, but we lack a precise predictive model to target this expensive therapy to those at highest risk for severe OM.

Independent risk factors for severe OM include renal dysfunction and melphalan dose, as we have previously shown among 381 myeloma patients receiving HD-MEL in which 75 % developed OM and 21 % developed severe OM [10]. We developed a model for severe OM using renal dysfunction and milligrams per kilogram melphalan dose as independent risk factors. However, significant differences in OM rates among patients with normal kidney function receiving the same milligrams per kilogram melphalan dose strongly suggest that additional factors such as genetic variability may play an important role in the pathogenesis of OM.

We herein report the results of an Institutional Review Board-approved comprehensive study to test our hypothesis that genetic variants play a role in susceptibility of multiple myeloma patients to chemotherapy-induced OM using genome-wide associations study (GWAS) together with clinically relevant factors for OM. The study included a homogenous population of 972 Caucasian patients with newly diagnosed multiple myeloma and at risk for OM as a result of therapy with HD-MEL.

Methods

Patients and treatment regimens

Data were collected from medical records on 972 Caucasian adults with newly diagnosed multiple myeloma cared for at the Myeloma Institute for Research and Treatment (MIRT) between March 1998 and September 2010. All except 134 patients were

enrolled in one of three consecutive clinical trials of Total Therapy (TT2, TT3, and TT4) [11–13]. All underwent HD-MEL-based ASCT and the data are related to the first transplant. To be transplant-eligible, patients were required to have good oral health. The Institutional Review Board approved the study; GWAS data available in dbGAP (phs000545.v1.p1).

The chemotherapy agents used in induction for each type of protocol are shown in Table 1. Following induction, patients received HD-MEL (140–200 mg/m²) based on body surface area (BSA), calculated according to Mosteller's formula [14]. MEL at 140 mg/m² was based on the age of >70 years. Patients were managed according to MIRT-predefined standards of care including oral antimicrobial prophylaxis with acyclovir, fluconazole, and fluoroquinolone throughout neutropenia [10]. Recombinant keratinocyte growth factor-1 prophylaxis was not used. Symptoms of OM were treated with topical agents, often in combination (aluminum hydroxide and magnesium hydroxide, diphenhydramine and/or viscous lidocaine) with the addition of opiates for severe pain.

The time period for the observation of OM began on the day stem cells were infused (day 0) and concluded on day + 21, thereby encompassing the days when patients are at risk for chemotherapy-induced mucositis. Clinicians (physicians, mid-level providers, and nurses) experienced in the management of patients with myeloma treated with HD-MEL conducted the patient assessments every other day, or daily as clinically indicated, until engraftment and resolution of complications.

Mucositis grading

Although mucositis grading was done prospectively by experienced nurses and physicians for all patients during their treatment, all patient files were reviewed again retrospectively for this study by two physicians and two nurses who individually graded mucositis based on the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for Gastrointestinal Events [15]. Any differences in grading were discussed and finalized for uniformity. Grades refer to the severity of mucositis with grade 1 = mild, grade 2 = moderate, grade 3 = severe, grade 4 = life-threatening, and grade 5 = death. We used grade 0 for no mucositis; grade 1 for mild symptoms like oral pain but intervention not indicated; grade 2 for moderate pain, interfering with oral intake, diet-modified; grade 3 for severe pain, interfering with oral intake, switched to liquid diet/medications switched to intravenous; and grade 4 hospitalized for severe pain requiring hospitalization, all medications intravenously, no oral intake.

Clinical risk factor data

Several baseline variables obtained immediately prior to the infusion of HD-MEL were evaluated including age, body mass index (BMI), BSA, weight, gender, type of treatment

Table 1 Chemotherapy agents received at the time of transplant in the Total Therapy protocols

Regimen type	Medication	Total dose received by treatment protocol		
		TT2	TT3	TT4
Induction	Cisplatin	30 mg/m ²	20 mg/m ²	20 mg/m ²
	Cyclophosphamide	1,400 mg/m ²	800 mg/m ²	800 mg/m ²
	Dexamethasone	160 mg	160 mg	160 mg
	Doxorubicin	25 mg/m ²	20 mg/m ²	10 mg/m ²
	Etoposide	80 mg/m ²	80 mg/m ²	80 mg/m ²
	Vincristine	0.5 mg	–	–
	Bortezomib	–	2 mg/m ²	2 mg/m ²
	Thalidomide	–	1,600 mg	1,600 mg
	Melphalan	–	–	20 mg/m ²
Conditioning	Melphalan	140–200 mg/m ²	140–200 mg/m ²	140–200 mg/m ²

m²: Based on body surface area (BSA) calculated according to Mosteller's formula

protocol, hematocrit, hemoglobin, renal function [estimated glomerular filtration rate (GFR)], serum albumin, C-reactive protein, melphalan dose received (in milligrams per kilogram), β 2-microglobulin (β 2-M), and myeloma isotype.

Genetic data

DNA for this study was obtained from the MIRT bank of germline DNA samples prepared from leukapheresis products. Genotyping was performed using the Illumina® Whole Genome Genotyping Infinium chemistry and HumanOmni-Quad BeadChip that contains over 1.1 million single-nucleotide polymorphisms (SNPs) and CNVs (Illumina, San Diego, CA). SNP genotypes were scored with the Genotyping Module of the Illumina GenomeStudio Data Analysis software. Individual samples with genotype call rates less than 98 % and SNPs with call rates less than 97 % were removed.

We randomly selected 2 % of the DNA samples to be plated in duplicate for quality control checks, and the average concordance rate for duplicate genotyping was 99.95 %. Furthermore, 953 DNA samples from the patient population of 972 patients (excludes 19 samples which were exhausted or of insufficient quantity) were sent to LGC Genomics for independent verification of genotypes using quantitative PCR (qPCR), and there was perfect concordance between the Illumina and LGC Genomics genotype calls at each of the significant SNPs. Of the 983,062 SNPs genotyped, there was sufficient information to conduct the statistical analysis for 892,589 SNPs in 972 patients, 619 patients with grades 0–1 OM, and 353 patients with grades 2–4 OM.

Statistical methods

The proportion of patients who developed grades 2–4 OM was estimated with the binomial proportion and its exact

confidence interval. The *t* test for independent samples and chi-square test were used to compare the patients who had grades 0–1 OM with those who had grades 2–4 OM with respect to baseline characteristics. Estimated GFR was used to assess renal function [16] for each SNP, and the Cochran-Armitage trend test [17, 18] was used to compare genotypes with respect to proportion of patients who developed grades 2–4 OM. The false discovery rate (FDR) approach [19] was used on the significance levels (*p* values) for the analyses to determine which were significantly associated with grades 2–4 OM at the overall 0.05 significance level. Because hemoglobin is highly correlated with hematocrit and body mass index and body surface area and body weight are highly correlated, only hematocrit and body surface area were used in the multivariate analysis. A stepwise regression model was used to find baseline clinical factors associated with OM risk; logistic regression models were used to evaluate the association of genotypes with OM after adjusting for clinical factors significantly associated with OM risk.

Results

The study population consisted of 972 Caucasian adults with newly diagnosed multiple myeloma who underwent melphalan-based HD-ASCT. Mean age was 57.65 years, 64 % were males, and all patients were Caucasian. Grades 2–4 OM developed in 36.3 % of patients (95 % confidence interval 33.3–39.4 %). Factors significantly (*p*<0.001) different by univariate analyses between patients with grades 0–1 and 2–4 OM were smaller BMI and BSA, lower weight, female gender, Total Therapy II protocol, higher albumin, renal dysfunction (lower estimated GFR), and higher milligrams per kilogram melphalan dose (Table 2). Females received higher doses of milligrams per kilogram melphalan

Table 2 Oral mucositis clinical risk factors

Baseline characteristics	All patients (N=972)	OM		
		Grades 0–1 (N=619)	Grades 2–4 (N=353)	Probability for comparing groups
Age in years, mean (SD)	57.65 (9.16)	57.81 (9.35)	57.36 (8.81)	0.460 ^a
Body mass index, mean (SD)	27.55 (5.10)	27.97 (5.01)	26.82 (5.18)	<0.001 ^a
Body surface area (m ²), mean (SD)	1.87 (0.23)	1.90 (0.24)	1.82 (0.21)	<0.001 ^a
Weight (kg), mean (SD)	81.11 (18.02)	83.27 (18.15)	77.32 (17.17)	<0.001 ^a
Gender				
Male (%)	63.68	69.47	53.54	<0.001 ^b
Female (%)	36.32	30.53	46.46	
Hematocrit (%), mean (SD)	33.40 (4.44)	33.65 (4.23)	32.97 (4.77)	0.027 ^a
Hemoglobin (g/dL), mean (SD)	11.01 (1.44)	11.09 (1.38)	10.89 (1.53)	0.037 ^a
Estimated GFR, mean (SD)	80.44 (28.40)	84.44 (23.28)	73.43 (30.58)	<0.001 ^a
Albumin (g/dL), mean (SD)	3.85 (0.50)	3.81 (0.47)	3.93 (0.53)	<0.001 ^a
C-reactive protein (mg/L), mean (SD)	9.36 (13.68)	9.02 (10.38)	9.96 (18.07)	0.373 ^a
Melphalan dose (mg/kg), mean (SD)	4.44 (0.79)	4.36 (0.79)	4.59 (0.78)	<0.001 ^a
β2-microglobulin, median (IQR)	3.43 (4.31)	2.95 (2.26)	4.26 (6.42)	0.091 ^a
Myeloma isotype				
FLC (%)	20.58	57.50	46.48	0.319 ^b
IgA (%)	22.53	65.75	34.25	
IgD (%)	0.72	71.43	28.57	
IgG (%)	54.01	64.76	35.24	
Other (%)	2.16	71.43	28.57	
Treatment protocol				
TT2 (%)	310 (31.89)	135 (43.55)	175 (56.45)	<0.001 ^b
TT, other (%)	528 (54.32)	393 (74.43)	135 (25.57)	
Non-TT (%)	134 (13.78)	91 (67.91)	43 (32.09)	

^a Based on *t* test for independent samples^b Based on chi-square test

(mean 4.72, SD 0.82) than men (mean 4.29, SD 0.73) ($p<0.001$, *t* test for independent samples), but there were no significant gender differences in type of treatment protocol nor in estimated GFR. BSA was not a significant baseline clinical factor when entered into a stepwise logistic regression model along with other clinical factors ($p>0.05$). The clinical factors associated with OM were type of treatment protocol ($p<0.001$), estimated GFR ($p<0.001$), melphalan dose ($p<0.001$), serum albumin ($p<0.001$), and gender ($p=0.002$). Concordance between observed and predicted levels of OM occurred in 46.5 % with type of treatment protocol, 70.9 % of the cases with the addition of estimated GFR, 72.8 % with the addition of melphalan dose (milligrams per kilogram), and 73.7 % with the addition of gender.

Of the 892,589 SNPs that were evaluated to determine if they were associated with oral mucositis, 110 SNPs met the overall 0.05 significance level using the false discovery rate approach (Table 3). None achieved significance at the 5×10^{-8} . Using logistic regression analyses, and after adjusting for type of treatment protocol, estimated GFR, melphalan

dose, serum albumin, and gender, each of these SNPs was assessed for association with oral mucositis. Because 99 of the SNPs were markers for gender, they were not included in the multivariate analysis.

The remaining 11 SNPs located on seven chromosomes were significantly associated with grades 2–4 OM, and odds ratios with 95 % confidence intervals indicate a significant difference between genotypes for all the SNPs with respect to incidence of grades 2–4 OM (Table 4). Concordance between observed and predicted levels of OM ranged from 74.6 to 75.5 % for those SNPs that were estimable ($p<.001$) and was 78.9 % when all 11 SNPs were included in the multivariate analysis (Table 5). There are three loci (near the genes matrix metalloproteinase 13 (MMP13), LINC00692, and DMRTA1/FLJ35282) with pairs of significant SNPs shown in Table 4, and these SNP pairs have high rates of linkage disequilibrium ($R^2>0.84$). Odds ratios demonstrated that homozygosity was positively associated with grades 2–4 oral mucositis. There were no significant gender differences between genotypes for any of the SNPs with respect to incidence of grades 2–4 OM.

Table 3 Frequency of single-nucleotide polymorphisms (SNPs) associated with grades 2–4 oral mucositis

Chromosome	Gene symbol or nearby gene symbol	SNP frequency
1	PLD5	3
	PPP1R12B	1
3	LINC00692	2
5	FBN2	1
	PTGER4	1
11	SHANK2	1
	MMP13	2
9	ALDH1A1	1
	DMRTA1	2
16	JPH3	1
17	DHRS7C	1
18	CEP192	1
XY	CD99	1
	CPXCR1	3
	PABPC5	9
	PCDH11X	58
	TGIF2LX	19
Y	XG	2
	TTY3	1

Clinical risk factors alone correctly predicted 43.6 % of grades 2–4 OM cases and 89 % of the grades 0–1 OM cases (Table 5). Adding individual SNPs to the model increased sensitivity of detecting grades 2–4 OM cases by only 2.1 % with little changes to the specificity in identifying grades 0–1 OM cases. When all 11 SNPs were added to clinical risk factors, sensitivity in detecting grades 2–4 OM increased to 52 %. Thus, the statistical model combining clinical risk factors and genetic variants correctly predicted 52 % of the patients with grades 2–4 oral mucositis and 86.9 % of the patients with grades 0–2 mucositis.

Discussion

Comparing groups with grades 0–1 vs grades 2–4 OM, our study revealed that 11 SNPs located on seven chromosomes were significantly associated with grades 2–4 OM. Six of these SNPs reside in introns of genes, two SNPs reside in a long terminal repeat region (LTR) and in a long interspersed repeated DNA (LINE), and three SNPs reside in large intergenic noncoding RNAs (lincRNAs) of the associated genes, and all may affect gene regulation. The position of the SNP in the LINE region may not be accurate due to its high homology (up to 87.7 % UCSC blat) to many other regions in the genome, and therefore, its exact location would

need to be verified by sequencing. LincRNAs are classified as noncoding RNAs (over 200 bases) and have been found to be involved in gene regulation using either *cis* or *trans* mechanisms [20]. It has also been established that SNPs in lincRNAs can affect their structural folding and binding of the RNA to its target and therefore influence gene activity [21]. We suspect that the mucositis-associated SNPs in these lincRNAs could affect expression of target genes, but this needs to be validated experimentally. Sonis [22] mapped 40 OM-associated SNPs to 29 genes; although some of the genes were associated ontologically with transcription regulation, RNA metabolic process regulation, and cell membrane integrity, none of the 29 genes were associated with OM in our study.

While type of treatment protocol and renal function represent the majority of risk predisposition, several of these genetic loci could have effects on OM development. In the Sonis model [23], the complex pathogenesis of chemotherapy-induced mucositis can be viewed as consisting of five inter-related phases: initiation, upregulation with generation of messengers, signaling and amplification, ulceration and inflammation, and healing.

It is plausible that the patients carrying different mucositis-associated SNPs may express variable levels of fibrillin 2 and matrix metalloproteinase 13 (MPP-13), and thus, they may exhibit different sensitivities to melphalan-induced OM. Fibrillin 2 encoded by *FBN2* is expressed in keratinocytes [24], is one of the components of the extracellular matrix, and has been shown to play an important role in the regulation of the bioavailability of TGF β in a tissue [25, 26]. Dietary supplementation of TGF β -2 reduced methotrexate-induced intestinal mucositis in rats [27], but the effects of TGF β -2 and TGF β -3 administration on chemotherapy-induced mucositis in humans have yet to be demonstrated [28, 29]. In contrast, a recent study showed that Smad7 can reduce radiation-induced OM by dampening TGF β signaling [30]. It remains to be determined whether patients with a different SNP rs10072361 exhibit a differential expression of fibrillin 2 and different TGF β activity in correlation with their susceptibility to melphalan-induced OM.

Additionally, it has been shown that expression of MMP13 is upregulated in oral epithelial cells during inflammation [31]. Given the role that MPP-13 plays in epithelial wound healing via regulating keratinocyte migration, angiogenesis, and inflammation [32, 33], patients with different alleles for SNPs (rs1940228 and rs948695) may exhibit different sensitivities to melphalan-induced OM if these alleles can affect the expression of MPP13.

All our patients received melphalan and cyclophosphamide (CTX) as part of their total therapy regimens [11–13]. Patients on the Total Therapy II protocol received a cumulative dose of 1,400 mg/m² of CTX for induction vs 400–800 mg/m² for patients on the other protocols, and more patients on Total Therapy II protocol had more severe OM. Dehydrogenases

Table 4 Single-nucleotide polymorphisms (SNPs) associated with grades 2–4 oral mucositis

Gene symbol or nearby gene symbol (chromosome location)	SNP	Genotype	All N (%)	Grades 2–4 oral mucositis N (%)	OR (95 % CI)
CPEB1/LINC00692 (3p24.2)	rs1426765	AA	773 (79.53)	251 (32.47)	AA vs AG, 0.45 (0.32, 0.65)
		AG	186 (19.14)	95 (51.08)	AA vs GG, 0.42 (0.13, 1.36)
		GG	13 (1.34)	7 (53.85)	AG vs GG, 0.92 (0.27, 3.09)
	rs6804277	AA	773 (79.53)	251 (32.47)	AA vs AG, 0.44 (0.30, 0.63)
		AG	184 (18.93)	95 (51.63)	AA vs GG, 0.65 (0.21, 1.99)
		GG	15 (1.54)	7 (46.67)	AG vs GG, 1.50 (0.48, 4.72)
FBN2 (5q23-q31)	rs10072361	AA	649 (67.39)	266 (40.99)	AA vs AG, 1.80 (1.29, 2.51)
		AG	280 (29.08)	82 (29.29)	AA vs GG, 6.42 (2.16, 19.07)
		GG	34 (3.53)	4 (11.76)	AG vs GG, 3.56 (1.18, 10.81)
ALDH1A1 (9q21.13)	rs1469167	AA	881 (90.64)	300 (34.05)	AA vs AG, 0.36 (0.22, 0.58)
		AG	90 (9.26)	52 (57.78)	AA vs GG (nonestimable)
		GG	1 (0.10)	1 (100)	AG vs GG (nonestimable)
DMRTA1/FLJ35282 (9p21.3)	rs62572481	CC	912 (93.83)	315 (34.54)	CC vs CT, 0.32 (0.18, 0.58)
		TC	59 (6.07)	37 (62.71)	CC vs TT (nonestimable)
		TT	1 (0.10)	1 (100)	TC vs TT (nonestimable)
	rs62572531	CC	1 (0.10)	1 (100)	CC vs TC (nonestimable)
		TC	60 (6.18)	38 (63.33)	CC vs TT (nonestimable)
		TT	910 (93.72)	314 (34.51)	TC vs TT, 3.26 (1.81, 5.84)
MMP13 (11q22.3)	rs1940228	AA	928 (95.57)	323 (34.81)	AA vs AG, 0.27 (0.13, 0.56)
		AG	43 (4.43)	30 (69.77)	
	rs948695	AA	925 (95.16)	320 (34.59)	AA vs AG, 0.25 (0.12, 0.49)
		AG	47 (4.84)	33 (70.21)	
JPH3 (16q24.3)	rs4843257	AA	220 (22.63)	104 (47.27)	AA vs AG, 1.55 (1.08, 2.21)
		AG	458 (47.12)	170 (37.12)	AA vs GG, 2.56 (1.70, 3.84)
		GG	294 (30.25)	79 (26.87)	AG vs GG, 1.66 (1.17, 2.34)
DHRS7C (17p13.1)	rs11078818	AA	29 (2.99)	17 (58.62)	AA vs AG, 1.37 (0.58, 3.26)
		AG	263 (27.09)	120 (45.63)	AA vs GG, 2.58 (1.11, 5.98)
		GG	679 (69.93)	216 (31.81)	AG vs GG, 1.88 (1.37, 2.60)
CEP192 (18p11.21)	rs12606033	AA	12 (1.23)	7 (58.33)	AA vs AG, 1.06 (0.29, 3.94)
		AG	220 (22.63)	106 (48.18)	AA vs GG, 2.10 (0.58, 7.59)
		GG	740 (76.13)	240 (32.43)	AG vs GG, 1.97 (1.41, 2.77)

such as aldehyde dehydrogenase 1A1 (ALDH1A1) may detoxify CTX [34, 35]; therefore, if patients with different alleles for SNP rs1469167 express variable levels of ALDH1A1, they could develop OM with different severities after CTX treatment. Finally, possible participation in CTX detoxification by DHRS7c, which also encodes a short-chain dehydrogenase/reductase [36], has yet to be determined. Therefore, the significance of SNP rs11078818 located in the intron region of DHRS7c in the development of OM in MM patients after chemotherapy remains to be investigated.

In our sample, females received significantly higher doses of melphalan than males and the incidence and severity of mucositis was significantly higher among females than among males ($p < 0.001$, Table 2); however, there were no gender differences in the genetic findings to account for the increased

risk of OM. We have previously shown that BSA dosing of melphalan results in a wide variation in milligrams per kilogram melphalan dose given, with females receiving the higher doses [10], and others found this similar variation [3]. Unfortunately, current dosing methods used in clinical practice understate the impact gender difference plays in drug metabolism. There is the need to recognize this more often, and more effort needs to be directed towards providing clinicians with tools to adjust doses accordingly.

Because multiple myeloma is more common among African-Americans, our findings from a sample of 972 Caucasians may not be applicable to the general multiple myeloma population. However, the homogeneity of our sample with regard to race is a strength of the study. Population stratification, a special confounder in disease association studies, occurs when

Table 5 Logistic regression model for OM: sensitivity to detect grades 2–4 and specificity to detect grades 0–1

Model components	Sensitivity (%) ^a	Specificity (%) ^b	Concordance (%) ^c
Clinical risk factors (CRFs) only	43.6	84.3	73.7
CRF+rs1940228 (gene MMP13)	43.9	85.0	74.6
CRF+rs948695 (gene MMP13)	44.5	85.0	74.9
CRF+rs4843257 (gene JPH3)	43.9	83.0	75.1
CRF+rs11078818 (gene DHRS7C)	44.8	86.4	75.1
CRF+rs12606033 (gene CEP192)	42.8	85.1	75.0
CRF+rs1426765 (gene LOC64506)	43.6	85.5	74.8
CRF+rs6804277 (gene LOC64506)	44.2	85.5	74.8
CRF+rs10072361 (gene FBN2)	45.7	83.8	75.5
CRF+rs1469167 (gene ALDH1A1)	43.3	86.3	74.9
CRF+SNP9-22707406 (gene DMRTA1)	45.3	84.6	74.7
CRF+SNP9-22735471 (gene DMRTA1)	45.6	84.8	74.8
CRF+All 11 SNPs	52.00	86.9	78.9

^a Proportion of patients with grades 2–4 oral mucositis predicted by the statistical model

^b Proportion of patients with grades 0–1 oral mucositis predicted by the statistical model

^c Proportion of patients whose oral mucositis was predicted by the model

allele frequency of the gene under study and a true risk factor for a disease in question vary considerably across subgroups of the population with a different risk for the disease. Therefore, the influence of population stratification on our results, if any, should be minor.

The current cohort of myeloma patients is the largest examined to date with little heterogeneity; all patients had newly diagnosed multiple myeloma, received a similar treatment regimen that included HD-MEL, and were cared for at a single institution. In addition, grading of mucositis was carefully evaluated by individuals trained in and experienced with mucositis assessment and was based on an accepted grading system (CTCAE version 4.0). Our findings may not be applicable to other patient populations treated with non-melphalan-based regimens. We nevertheless identified 11 SNPs statistically significant at an FDR of 0.05 which remained significant ($p < 0.001$) when included as covariates in a logistic regression model of multiple baseline clinical characteristics. That none of the mucositis-associated SNPs reached the most stringent levels of genome-wide multiplicity adjustment (typically, 5×10^{-8}) [37] is likely related to sample size limitations. In a separate study, we plan to validate these results in a different sample of patients with chemotherapy-induced OM.

In conclusion, we have discovered previously unreported genetic associations for OM risk in a locus that is plausible for effect given its known role in the inflammation cascade. Although these findings may not be readily applicable clinically, they help provide some insight into the pathophysiology of OM. Patients carrying different mucositis-associated SNP alleles may express variable levels of fibrillin 2, MMP13, ALDH1A1, and/or DHRS7c and thus exhibit altered

sensitivity to OM induced by melphalan and other chemotherapeutic agents such as CTX. We suspect that the mucositis-associated SNPs could affect expression of adjacent genes through the noncoding RNA and this pathway may eventually represent a novel drug target for the prevention of this devastating condition. This hypothesis will be tested in our future studies. Our clinical findings complement earlier reports of genetic findings and may help us understand the pathophysiology of OM.

Conflict of interest The authors have no conflict of interest to report. Author/COI Disclosure Forms were submitted along with the manuscript.

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