

ORIGINAL ARTICLE

Genetic variants of *SDCCAG8* and *MAGI2* in mitosis-related pathway genes are independent predictors of cutaneous melanoma-specific survival

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Abstract

Mitosis is a prognostic factor for cutaneous melanoma (CM), but accurate mitosis detection in CM tissues is difficult. Therefore, the 8th Edition of the American Joint Committee on Cancer staging system has removed the mitotic rate as a category criterion of the tumor T-category, based on the evidence that the mitotic rate was not an independent prognostic factor for melanoma survival. As single-nucleotide polymorphisms (SNPs) have been shown to be potential predictors for cutaneous melanoma-specific survival (CMSS), we investigated the potential prognostic value of SNPs in mitosis-related pathway genes in CMSS by analyzing their associations with outcomes of 850 CM patients from The University of Texas MD Anderson Cancer Center in a discovery dataset and validated the findings in another dataset of 409 CM patients from the Harvard University Nurses' Health Study and Health Professionals Follow-up Study. In both datasets, we identified two SNPs (*SDCCAG8* rs10803138 G>A and *MAGI2* rs3807694 C>T) as independent prognostic factors for CMSS, with adjusted allelic hazards ratios of 1.49 (95% confidence interval = 1.17-1.90, $P = .001$) and 1.45 (1.13-1.86, $P = .003$), respectively. Furthermore, their combined unfavorable alleles also predicted a poor survival in both discovery and validation datasets in a dose-response manner ($P_{\text{trend}} = .0006$ and $.0001$, respectively). Additional functional analysis revealed that both *SDCCAG8* rs10803138 A and *MAGI2* rs3807694 T alleles were associated with elevated mRNA expression levels in normal tissues. Therefore, these findings suggest that *SDCCAG8* rs10803138 G>A and *MAGI2* rs3807694 C>T are independent prognostic biomarkers for CMSS, possibly by regulating the mRNA expression of the corresponding genes involved in mitosis.

KEYWORDS

cutaneous melanoma, mitosis, single-nucleotide polymorphism, survival

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1 | INTRODUCTION

Cutaneous melanoma (CM) is a highly aggressive skin cancer with an estimated 106 110 new cases and 7180 deaths in 2021 in the United States.¹ As CM-specific survival (CMSS) varies greatly despite currently available therapies,² the identification of accurate prognostic factors for CMSS could aid in tailoring clinical treatment decisions for different subgroups of CM patients.

Mitosis has long been considered a key marker of cell growth, and an elevated mitotic rate in CM tumor tissue generally suggests that the tumor is highly aggressive with a worse prognosis.³ Previous studies showed the benefits of mitosis assessment on survival and management of CM patients⁴⁻⁷; however, the mitotic rate was hand-calculated under the microscope by physicians and pathologists, introducing some level of uncertainty⁸; furthermore, an accurate detection of mitosis in thin, small CM tissue samples has been proven difficult.⁹ Therefore, although the previous melanoma staging manual of the American Joint Committee on Cancer (AJCC) endorsed the mitotic rate, thickness, and ulceration as prominent adverse prognostic factors for CMSS, the latest edition has removed the mitotic rate as a criterion for staging, because the mitotic rate was not an independent predictor for CMSS,¹⁰ but rather the thin tumor tissues with a mitotic rate of ≥ 1 mitosis per mm^2 had a better prognosis than thicker tumor tissues (without mitotic figures) in CM subgroup analyses.^{10,11} Therefore, identifying additional accurate indicators of mitoses to be used in predicting CMSS may enrich the melanoma staging system, improve prognostic estimates for CM, and provide a better guide for CM patient treatment.

Previous studies demonstrated that single-nucleotide polymorphisms (SNPs) were associated with both tumor development and patient survival,^{12,13} implying the possibility of specific genetic variants as prognostic factors for CMSS. In fact, some specific SNPs may affect the functions of target pathogenic molecules of melanoma, consequently affecting melanoma disease course and outcomes.¹⁴⁻¹⁶ Although several genome-wide association studies (GWASs) have identified some susceptibility loci for CM,¹⁷⁻¹⁹ few SNPs have been reported to be associated with CMSS at the GWAS level, because those GWAS studies focused strictly on SNPs with the most-significant *P*-values, using a highly stringent correction for the multiple testing, without any recourse to the underlying molecular mechanisms, therefore limiting the opportunities for further functional analysis and prognostic estimate of clinical outcomes.^{20,21} However, a hypothesis-driven post-GWAS strategy has some advantages by using available genotyping data from previously published GWAS datasets in identifying functional genetic variants in targeted biological pathway genes and clarify their associations with CMSS at a pathway level.²² Therefore, to better understand the prognostic value of mitosis for CMSS, we hypothesize that genetic variants of mitosis-related pathway genes are associated with CMSS, and we tested this hypothesis by using publicly available CM GWAS datasets.

2 | MATERIALS AND METHODS

2.1 | Study populations

The discovery dataset comprised genotyping and survival data on 858 patients derived from The University of Texas MD Anderson Cancer Center (MDACC) CM GWAS study (dbGaP accession #: phs000187.v1.p1). All patients were recruited from a hospital-based case-control study of non-Hispanic White CM patients. In contrast, the replication dataset used genotyping and survival data on an additional 409 participants from CM GWAS datasets in the Harvard University Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS), in which incident CM cases among Whites were collected during the follow-up over 14 years. Definition of the participants and methods of data collection for both discovery and validation datasets have been published in detail elsewhere.^{18,23} All the participants provided a written-informed consent under a protocol approved by the Institutional Review Boards of MDACC, Brigham and Women's Hospital, and Harvard TH Chan School of Public Health, and those of participating registries as required.

2.2 | Gene selection

Based on Molecular Signatures Database of the Gene Set Enrichment Analysis (GSEA) website (<http://software.broadinstitute.org/gsea/msigdb/search.jsp>), we comprehensively identified 226 mitosis-related pathway genes located only on the autosomes (Table S1). As females have two copies of the X chromosome and there are no recognized statistical analytic methods established for sex-specific analysis, we did not include genes on the X chromosome in the final analysis.

2.3 | SNP genotyping

Details involving genomic DNA extracting and genotyping data obtaining from the MDACC, NHS, and HPFS datasets are presented in Document S1.

2.4 | Statistical methods

We first assessed associations between all available SNPs in mitosis-related pathway genes and CMSS in a single-locus Cox proportional hazards regression analysis. Then, we performed multivariate Cox proportional hazards regression analyses with adjustment for available covariates in the MDACC dataset (including age, sex, Breslow thickness, mitotic rate, distant/regional metastasis and ulceration); however, in the replication (NHS/HPFS) dataset, the only available covariates for adjustment were age and sex. We used the less stringent Bayesian False Discovery Probability (BFDP) for multiple testing correction, because the vast majority of the SNPs under investigation is in high linkage disequilibrium (LD) and we intended to

identify the functional SNPs with such a relaxed correction method. We subsequently evaluated cumulative effects of identified SNPs via Kaplan-Meier (KM) survival curves, also showing the associations between CM survival probability and combined alleles. The receiver operating characteristic (ROC) curve and time-dependent area under the curve (AUC) were calculated to predict the effects of both clinical and genetic variables on CMSS. To evaluate the genotype-phenotype correlation between genotypes of identified SNPs and mRNA expression levels of their genes, we performed expression quantitative trait loci (eQTL) analyses using the data from the genotype-tissue expression (GTEx) project and 1000 Genomes project. Finally, we assessed the association between the mRNA expression levels and CM survival using KM analysis (<https://www.proteinatlas.org>) with data from The Cancer Genome Atlas (TCGA) database. Other details are presented in Document S1.

3 | RESULTS

3.1 | Baseline characteristics of study populations

Baseline characteristics of CM patients from MDACC, NHS, and HPFS datasets are described in Table S2. MDACC CM patients were between 17 and 94 y of age (mean 52.4 ± 14.4 y) at diagnosis, of whom 57.8% were men, and 82.6% had a stage I/II disease; the median follow-up time was 81.1 mo, and 11.1% died from CM. NHS/HPFS CM patients were between 34 and 87 y of age (mean 61.1 ± 10.8 y) at diagnosis, of whom 33.7% were men; the median follow-up time was 179.0 mo, and the mortality rate was 11.5%. There was no evidence of associations between principal components and CM survival in MDACC, NHS, or HPFS datasets; therefore, there was no need for further adjustment.

3.2 | Associations between SNPs in mitosis-related pathway genes and CMSS in MDACC and NHS/HPFS datasets

Overall flowchart of the present study is shown in Figure 1. We first assessed the associations between all 25 631 acquired (4361 genotyped and 21 270 imputed) SNPs in 226 mitosis-related pathway genes with CMSS in the MDACC dataset, and we found that 947 SNPs were associated with CMSS ($P < .05$) in an additive model after multiple test correction (BFDP < 0.8). In validation using the NHS/HPFS dataset, only 42 SNPs remained notably significant. These 42 SNPs are located in four genes, ie, 37 SNPs in *SDCCAG8*, two SNPs in *CCND3*, 2 SNPs in *DSCC1*, and one SNP in *MAGI2* (Table S3).

3.3 | Two independent SNPs predict CMSS

To identify independent SNPs associated with CMSS, we first performed stepwise multivariate Cox regression analyses and then LD

analysis to assess the effects of 42 validated SNPs on CMSS in the MDACC dataset (but not in the NHS/HPFS dataset not containing the same clinical covariates as the MDACC dataset). Four SNPs (rs10803138, rs9394852, rs3807694 and rs62526623) in four genes (*SDCCAG8*, *CCND3*, *MAGI2* and *DSCC1*) remained significantly associated with CMSS ($P < .05$) in the presence of clinical covariates. After we expanded this prediction model with adjustment for an additional 40 previously reported SNPs in the MDACC GWAS dataset, we found that two SNPs (*SDCCAG8* rs10803138 G>A and *MAGI2* rs3807694 C>T) remained independently and significantly associated with CMSS ($P = .010$ and $.016$, respectively) (Table 1). The meta-analysis of these two independent SNPs in each dataset is shown in Table 2 without heterogeneity across datasets.

Furthermore, as shown in Table 3, we noticed that *SDCCAG8* rs10803138 A and *MAGI2* rs3807694 T alleles were both identified as prognostic risk alleles for CMSS in the MDACC dataset ($P_{\text{trend}} = .017$ and $.032$, respectively) with similar results in the NHS/HPFS dataset ($P_{\text{trend}} = .033$ and $.032$, respectively) and the combined MDACC and NHS/HPFS dataset ($P_{\text{trend}} = .018$ and $.008$, respectively) (Table 3). All of the identified SNPs in the present study are depicted in a Manhattan plot (Figure S1), and regional association plots for the two SNPs are displayed in Figure S2.

3.4 | Combined risk alleles of the two independent CMSS-associated SNPs

To investigate the joint effect of the two independent SNPs on CMSS, we combined their risk alleles (ie, *SDCCAG8* rs10803138 A and *MAGI2* rs3807694 T alleles) into a genetic score; because the combination of genotypes from two SNPs led to a loss of power due to a small number of events in each category at the individual level, using a genetic risk score with summing up the number of risk alleles had a better discriminatory value at the patient population level, which reflects population risk of the patients who would have one (heterozygous) of the two different alleles (homozygous). Patients in each dataset were categorized into four groups (ie, 0, 1, 2, and 3-4) based on the number of risk alleles (NRAs), and the trend tests in each dataset all revealed a significant risk-allele dose-response effect on survival. Specifically, an increased NRAs was associated with a worse survival in the MDACC dataset ($P_{\text{trend}} < .001$), the NHS/HPFS dataset ($P_{\text{trend}} = .002$) and combined MDACC and NHS/HPFS dataset ($P_{\text{trend}} < .001$) after adjustment for available covariates (Table 3).

Furthermore, we dichotomized all CM patients into two groups: 0-1 or 2-4 NRAs. As shown in Table 3, compared with 0-1 NRAs group, the 2-4 NRAs group had a significantly worse CMSS in the MDACC dataset (HR = 2.07; 95% CI = 1.37-3.13, $P < .001$), in the NHS/HPFS dataset (HR = 2.53; 95% CI = 1.40-4.54, $P = .002$), and the combined MDACC and NHS/HPFS dataset (HR = 1.91; 95% CI = 1.36-2.65, $P < .001$). In addition, we also used KM survival

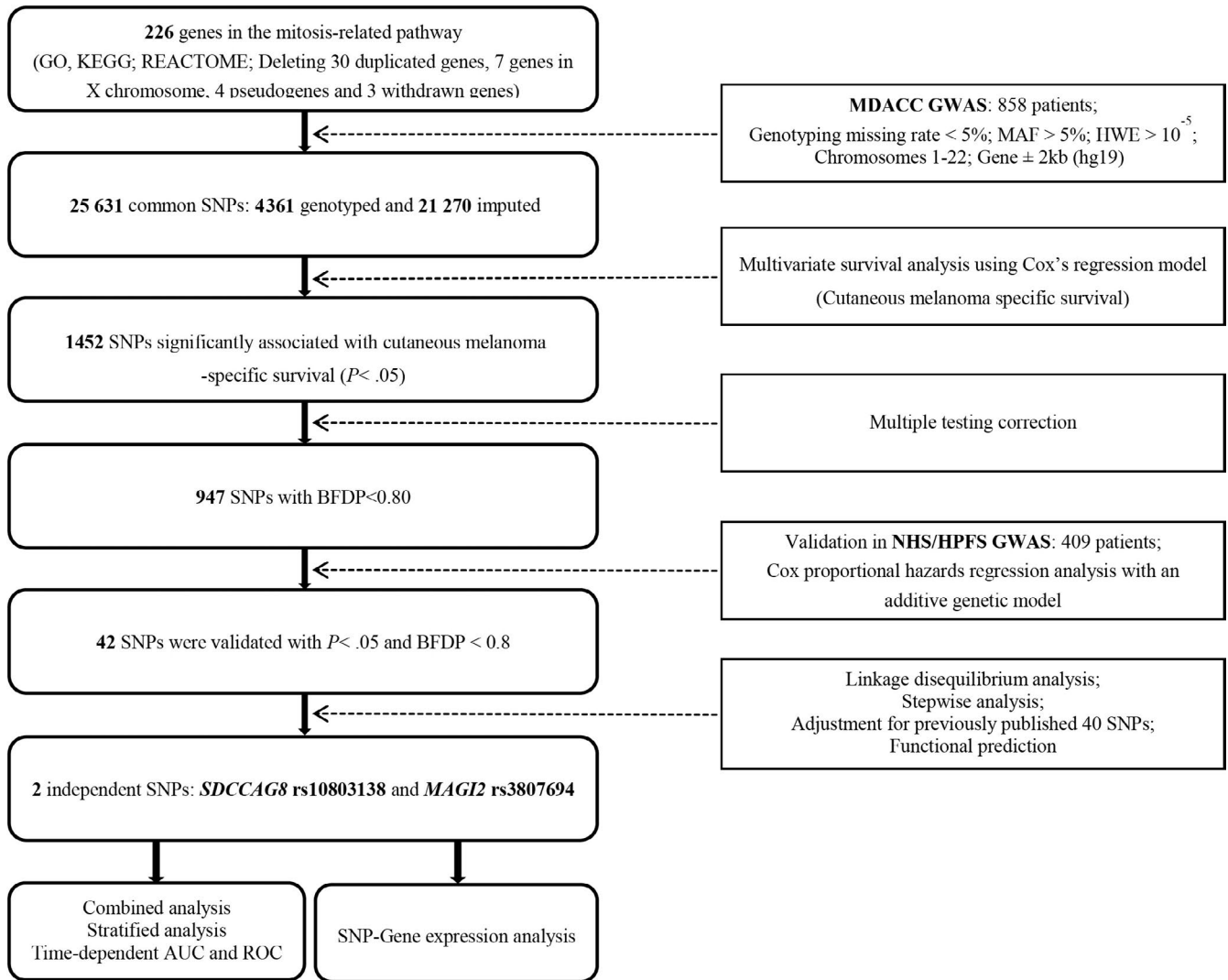


FIGURE 1 Flowchart of the study. AUC, area under the curve; BFDP, Bayesian false-discovery probability; GWAS, genome-wide association study; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; MDACC, The University of Texas MD Anderson Cancer Center; NHS/HPFS, the Nurses' Health Study/Health Professionals Follow-up Study; ROC, receiver operating characteristic; SNP, single-nucleotide polymorphism

curves to display the associations between NRAs and CMSS (Figure 2A-C).

3.5 | Stratified analysis for the effect of NRAs on CMSS

To assess whether the effects of NRAs on CMSS were modified by other clinical covariates, we performed stratified analysis in both MDACC and NHS/HPFS datasets. Compared with CM patients with 0-1 NRAs, survival was significantly poorer among CM patients with 2-4 NRAs, except for the subgroup aged ≤ 50 and having Breslow thickness ≤ 1 mm in the MDACC dataset and the subgroup of men aged ≤ 50 in the NHS/HPFS dataset. The statistics showed no interactions among the subgroups of each covariate (Table S4). In particular, we noticed that no interactions between the NRAs and mitosis rate.

3.6 | ROC curves and time-dependent AUC of the two independent SNPs for CMSS prediction

To further evaluate predictive value of the two independent SNPs, we constructed time-dependent AUC and ROC curves for CM patients in the presence of available clinical covariates. In the MDACC dataset, although the time-dependent AUC in the model with clinical variables increased from 85.74% to 86.83% when risk alleles were added, the predictive performance of 5-y CMSS ROC curves was not significantly improved ($P = .108$) (Figure S3A,B). However, the prediction performance of 5-y CMSS ROC curves were dramatically improved after the risk alleles were added to the model for stages I CM only; and the time-dependent AUC significantly increased from 86.83% to 92.73% with clinical variables as classifiers ($P = .023$) (Figure S3C,D). In the NHS/HPFS dataset, the predictive performance of 5-y CMSS ROC curves in the model with demographic covariates (ie, age and sex) was also

TABLE 1 Two independent SNPs identified by multivariate Cox proportional hazards regression analysis among the selected variables with adjustment for previously published survival-associated SNPs in the MDACC melanoma GWAS dataset

Variables ^a	Category ^b	Frequency	HR (95% CI) ^a	P ^a	HR (95% CI) ^c	P ^c
Age	≤50/>50	371/487	1.02 (1.00-1.04)	.032	1.04 (1.02-1.06)	< .001
Sex	Female/male	362/496	1.70 (1.06-2.73)	.029	1.19 (0.71-2.00)	.505
Regional/distant metastasis	No/yes	709/149	4.28 (2.76-6.63)	< .001	14.67 (7.87-27.34)	< .001
Breslow thickness (mm)	≤1/>1	347/511	1.18 (1.12-1.24)	<.001	1.23 (1.14-1.33)	< .001
Ulceration	No/yes	681/155	2.49 (1.61-3.86)	< .001	3.47 (2.02-5.78)	< .001
Mitotic rate (mm ²)	≤1/>1	275/583	2.49 (1.20-5.15)	.014	2.43 (1.07-5.54)	.035
SDCCAG8 rs10803138 G>A	GG/GA/AA	504/300/54	1.39 (1.02-1.90)	.036	1.70 (1.13-2.54)	.010
MAGI2 rs3807694 C>T	CC/CT/TT	325/404/129	1.38 (1.02-1.86)	.038	1.59 (1.09-2.32)	.016

Abbreviations: CI, confidence interval; HR, hazards ratio; MDACC, The University of Texas MD Anderson Cancer Center; SNP, single-nucleotide polymorphism.

^aStepwise analysis included age, sex, regional/distant metastasis, Breslow thickness, ulceration, mitotic rate and 42 validated SNPs.

^bThe “category/” was used as the reference.

^cForty published SNPs were used for post-stepwise adjustment: rs1175649, rs1124379, rs10916352, rs6707820, rs6750552, rs6785564, rs2306574, rs11551405, rs1718404, rs12512631, rs788935, rs32579, rs3734398, rs7826362, rs10090371, rs7850212, rs3851552, rs10882807, rs61873997, rs35748949, rs11018104, rs7944031, rs11037684, rs508485, rs7933369, rs11225163, rs1990330, rs7953425, rs2342924, rs10846684, rs206118, rs10492396, rs3752447, rs2596191, rs782917, rs17204952, rs62068372, rs72635537, rs7253062, rs3918251.

dramatically improved by adding the risk alleles ($P = 5.68 \times 10^{-5}$), and the time-dependent AUC increased from 54.05% to 78.76% (Figure S3E,F). Finally, in the combined MDACC and NHS/HPFS dataset, the predictive performance of 5-y CMSS ROC curves in the model with demographic variables (ie, age and sex) was also significantly improved by adding the risk alleles ($P = .024$), and time-dependent AUC increased from 63.60% to 68.21% (Figure 2D,E). We also evaluated 10-y CMSS ROC curves in the MDACC dataset, but the predictive performance of 10-y CMSS ROC curves was not significantly improved (data not presented).

3.7 | Functional predictions of the two independent SNPs

To identify biological functions of the two independent SNPs associated with CMSS, we explored SNP-related genomics data using online bioinformatics tools (HaploReg, <https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>). We noticed that the SDCCAG8 rs10803138 G>A change may disturb protein motifs; similarly, other identified significant SNPs showing a high LD ($r^2 \geq .8$) with rs10803138 in the present study may also be involved in regulating the expression of histone in specific regions, DNase expression, and protein binding. In addition, the MAGI2 rs3807694 C>T change was also predicted to disturb protein motifs (Table S3). By extracting data from the Encyclopedia of DNA Elements (ENCODE) project, we found that rs10803138 was probably located on the H3K4Me1 and H3K27Ac motifs, while rs3807694 was probably located on the H3K4Me1 motif (Figure S4). These findings strongly imply that the two independent SNPs may disturb their gene expression by transcriptional regulation.

3.8 | Two independent SNPs regulate their corresponding mRNA expression

To further explore molecular mechanisms underlying the associations between the two independent SNPs and CMSS, we established correlations between risk alleles of the two independent SNPs and their corresponding mRNA expression levels by eQTL analysis. In RNA-Seq data of lymphoblastoid cell lines from 373 European descendants (obtained from the 1000 Genomes Project), the rs10803138 A allele showed a significant correlation with increased expression levels of SDCCAG8 mRNA in all additive, dominant, and recessive models ($P < .001$, $P = .007$ and $P = .002$, respectively) (Figures 2F and S5A,B); however, there was no correlation between the rs3807694 T allele and expression levels of MAGI2 mRNA in any of the three genetic models (Figure S5C-E). Additionally, we performed eQTL by extracting data from the GTEx Project. The results showed that the rs10803138 A allele was significantly associated with increased expression levels of SDCCAG8 mRNA in normal tissues from sun-exposed lower leg skin ($P = 3.9 \times 10^{-3}$), unexposed suprapubic skin ($P = 5.86 \times 10^{-3}$) and whole blood ($P = 1.74 \times 10^{-7}$) (Figure 2G), all these were consistent with findings from the 1000 Genomes Project. However, the rs3807694 T allele was significantly correlated with higher expression levels of MAGI2 mRNA in normal tissues from sun-exposed lower leg skin ($P = 4.74 \times 10^{-3}$) (Figure 2H) but not in normal tissues from unexposed suprapubic skin ($P = .720$) (Figure S5F).

Finally, we evaluated the mRNA expression levels of SDCCAG8 and MAGI2 in 104 primary CM tissues and 368 metastatic CM tissues available from TCGA database (data obtained from <http://ualcan.path.uab.edu/index.html>). As shown in Figure S6A,C, mRNA expression levels of SDCCAG8 and MAGI2 were both significantly higher in metastatic CM tissues ($P = 4.73 \times 10^{-7}$ and $P = 4.62 \times 10^{-4}$,

TABLE 2 Meta-analysis of the two independent SNPs in mitosis-related pathway genes identified from two previously published melanoma GWAS datasets

SNP	Allele ^a	Discovery-MDACC (n = 858)			Validation NHS/HPFS (n = 409)			Combined-meta-analysis (n = 1267)					
		EAF	HR (95% CI) ^b	P ^b	BFDp ^c	EAF	HR (95% CI) ^d	P ^d	BFDp ^c	P _{het}	I ^b	HR (95% CI) ^e	P ^e
rs10803138 ^f	G>A	0.24	1.45 (1.07-1.98)	.017	0.725	0.26	1.57 (1.04-2.38)	.033	0.779	.760	0	1.49 (1.17-1.90)	.001
rs3807694 ^g	C>T	0.39	1.39 (1.03-1.87)	.032	0.792	0.40	1.58 (1.04-2.41)	.031	0.779	.630	0	1.45 (1.13-1.86)	.003

Abbreviations: BFDp, Bayesian false-discovery probability; CI, confidence interval; EAF, effect allele frequency; GWAS, genome-wide association study; HR, hazards ratio; MDACC, The University of Texas MD Anderson Cancer Center; NHS/HPFS, the Nurses' Health Study/Health Professionals Follow-up Study; P_{het}, P value for heterogeneity by Cochran's Q test; SNP, single-nucleotide polymorphism.

^aReference allele>effect allele.

^bAdjusted for age, sex, Breslow thickness, distant/regional metastasis, ulceration, and mitotic rate in an additive genetic model.

^cBFDp was used for multiple test correction with detected a highest HR of 2.0 and a prior probability of .1.

^dAdjusted for age and sex in an additive genetic model.

^eMeta-analysis in a fix-effects model.

^fImputed SNP in the MDACC GWAS dataset.

^gGenotyped SNP in the MDACC GWAS dataset.

respectively) than in primary CM tissues. Furthermore, we found

that a higher expression level of *SDCCAG8* mRNA was significantly associated with a worse CM survival, as shown by the KM survival curve ($P = .024$) (data obtained from The Human Protein Atlas, www.proteinatlas.org) (Figure S6B). However, the expression level of *MAGI2* mRNA was not significantly associated with CM survival ($P = .220$) (data obtained from The Human Protein Atlas, www.proteinatlas.org) (Figure S6D).

4 | DISCUSSION

In the present study, we explored the associations between 25 631 SNPs of 226 mitosis-related pathway genes and CMSS using available genotyping and data of 1267 CM patients from two reported CM GWAS datasets. We identified two SNPs (*SDCCAG8* rs10803138 G>A and *MAGI2* rs3807694 C>T) that were independently associated with CMSS. In addition, we found that the *SDCCAG8* rs10803138 A allele was significantly associated with an increased mRNA expression of the gene, while the *MAGI2* rs3807694 T allele was significantly associated with a higher mRNA expression of the gene. Furthermore, our results revealed that the mRNA expression of *SDCCAG8* and *MAGI2* were both increased in CM metastatic tissues, and a higher expression level of *SDCCAG8* mRNA was significantly associated with a poorer survival in CM patients.

The presence of mitoses, as well as tumor thickness and distant metastases, was considered a strong prognostic factor for CM.²⁴⁻²⁶ The seventh edition of AJCC recommended that the mitotic rate be used as a prognostic variable to stratify the T-category in T1 melanoma.²⁷ However, recent results on prognostic value of the mitotic rate have been controversial. In the AJCC updated edition, therefore, the mitotic rate was demonstrated not to be a valid independent prognostic variable in a multivariate analysis for predicting CMSS.¹⁰ This inconsistency may be due not only to the misuse of mitotic rate count as a cut-off dichotomous variable, but it is also rooted in the difficulty of accurately measuring mitosis rate in CM tumor tissue.^{8,28-31} In the present study, we investigated the prognostic value of genetic surrogates for mitosis in CM by analyzing associations between SNPs in mitosis-related pathway genes and CMSS, instead of using direct assessment for the mitosis rate in CM tissues. Although databases used in the present study included limited records of 1267 Caucasian CM patients, we identified two independent SNPs as independent prognostic determinants of CMSS in a multivariate analysis with adjustment for available multi-clinical covariates as well as 40 previously published survival-associated SNPs. As the two independent SNPs identified in the present study were determined to have no linkage to clinical covariates, these SNPs could form a new variable for determining prognosis/survival, if validated by other investigators. In addition, we have evaluated the correlation between the NRAs of two SNPs and mitotic rate in the MDACC dataset (data not presented). We found that both rs10803138 G>A and rs3807694 C>T were not correlated with the mitotic rate in the MDACC dataset ($P = .085$ and $.376$, respectively); therefore, these two SNPs may serve as a new prognostic biomarker

TABLE 3 Associations between the two identified independent SNPs in mitosis-related pathway genes and CMSS of patients in the MDACC dataset, the NHS/HPFS dataset, and the combined dataset

Genotype	MDACC (n = 858)				NHS/HPFS (n = 409)				MDACC + NHS/HPFS (n = 1267)			
	Frequency		Multivariate analysis ^a		Frequency		Multivariate analysis ^b		Frequency		Multivariate analysis ^c	
	All	Death (%)	HR (95% CI)	P	All	Death (%)	HR (95% CI)	P	All	Death (%)	HR (95% CI)	P
SDCCAG8 rs10803138 G>A												
GG	504	51 (10.12)	1.00		225	21 (9.33)	1.00		729	72 (9.88)	1.00	
GA	300	36 (12.00)	1.67 (1.07-2.60)	.024	153	20 (13.07)	1.42 (0.77-2.62)	.269	453	56 (12.36)	1.29 (0.91-1.83)	.155
AA	54	8 (14.81)	1.81 (0.85-3.85)	.126	31	7 (22.58)	2.65 (1.13-6.25)	.026	85	15 (17.65)	1.92 (1.10-3.35)	.022
Trend test				.017				.033				.018
MAGI2 rs3807694 C>T												
CC	325	27 (8.31)	1.00		140	13 (9.29)	1.00		465	40 (8.60)	1.00	
CT	404	50 (12.38)	1.43 (0.88-2.33)	.147	208	23 (11.06)	1.29 (0.65-2.55)	.466	612	73 (11.93)	1.37 (0.93-2.01)	.113
TT	129	18 (13.95)	1.92 (1.04-3.52)	.036	61	12 (19.67)	2.53 (1.14-5.60)	.022	190	30 (15.79)	1.91 (1.19-3.06)	.008
Trend test				.032				.032				.008
Number of combined risk alleles ^d												
0	187	16 (8.56)	1.00		74	4 (5.41)	1.00		261	20 (7.66)	1.00	
1	366	33 (9.02)	1.21 (0.64-2.27)	.559	161	14 (8.70)	1.56 (0.51-4.73)	.436	527	47 (8.92)	1.19 (0.71-2.02)	.507
2	225	34 (15.11)	2.23 (1.19-4.18)	.013	141	24 (17.02)	3.37 (1.17-9.72)	.025	366	58 (15.85)	2.13 (1.28-3.53)	.004
3-4	80	12 (15.00)	2.78 (1.27-6.09)	.011	33	6 (18.18)	4.10 (1.16-14.57)	.029	113	18 (15.93)	2.25 (1.19-4.26)	.013
Trend test				<.001				.002				<.001
0-1	553	49 (8.86)	1.00		235	18 (7.66)	1.00		788	67 (8.50)	1.00	
2-4	305	46 (15.08)	2.07 (1.37-3.13)	<.001	174	30 (17.24)	2.53 (1.40-4.54)	.002	479	76 (15.87)	1.91 (1.36-2.65)	<.001

Abbreviations: CI, confidence interval; CMSS, cutaneous melanoma-specific survival; HR, hazards ratio; MDACC, The University of Texas MD Anderson Cancer Center; NHS/HPFS, the Nurses' Health Study/Health Professionals Follow-up Study; SNP, single-nucleotide polymorphism.

^aAdjusted for age, sex, Breslow thickness, distant/regional metastasis, ulceration, and mitotic rate in Cox models in MDACC study.

^bAdjusted for age and sex in Harvard study.

^cAdjusted for age and sex in the combined dataset of the MDACC and Harvard studies.

^dRisk alleles include SDCCAG8 rs10803138 A allele and MAGI2 rs3807694 T allele.

for CMSS, but they may not be a surrogate of the mitosis rate in the

MDACC dataset.

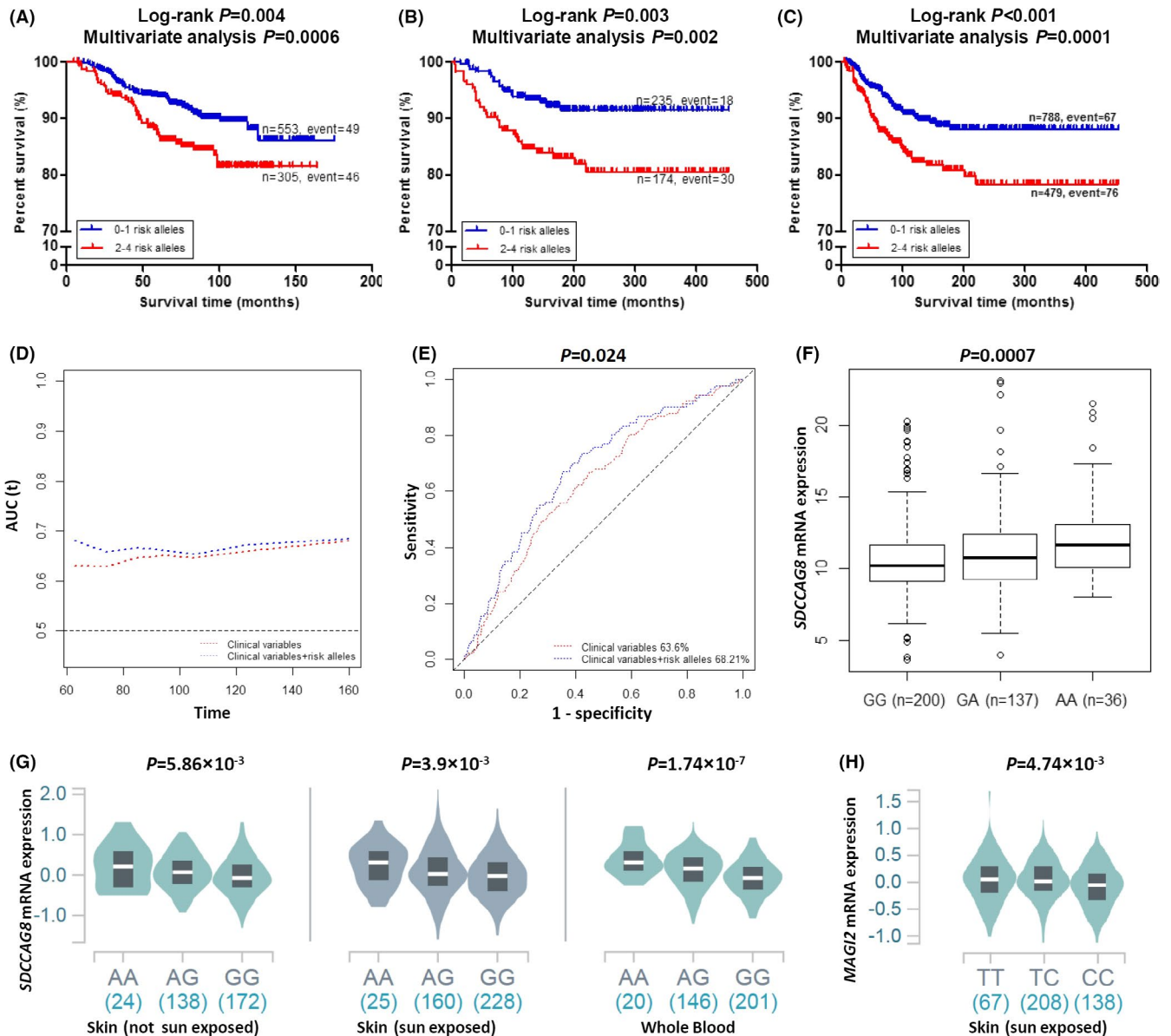


FIGURE 2 Two independent SNPs in mitosis-related pathway genes predict cutaneous melanoma survival and eQTL analysis for them. Kaplan-Meier survival curves of combined risk alleles of *SDCCAG8* rs10803138 and *MAGI2* rs3807694 on CMSS: dichotomized 0-1 risk-allele group and 2-4 risk alleles group in the MDACC dataset (A), the NHS/HPFS dataset (B) and combined MDACC and NHS/HPFS dataset (C). The time-dependent AUC estimation based on clinical variables plus risk alleles in combined MDACC and NHS/HPFS dataset (D). The 5-y CMSS prediction by ROC curve based on clinical variables plus risk alleles in combined MDACC and NHS/HPFS dataset (E). The correlation of rs10803138 genotypes and *SDCCAG8* mRNA expression in an additive model from the 1000 Genomes Project (F). The correlation of rs10803138 and *SDCCAG8* mRNA expression in skin tissues and whole blood samples from the GTEx (G). Correlation of rs3807694 and *MAGI2* mRNA expression levels in skin tissues from the GTEx (H). AUC, area under the curve; CMSS, cutaneous melanoma-specific survival; eQTL, expression quantitative trait loci; GTEx, Genotype-Tissue Expression project; HPFS, the Health Professionals Follow-up Study; MDACC, The University of Texas MD Anderson Cancer Center; NHS, the Nurses' Health Study; ROC, receiver operating characteristic; SNP, single-nucleotide polymorphism

In the present study, we found that the predictive performances of 5-y ROC curves and time-dependent AUC were significantly improved by adding risk alleles of the two significant SNPs to the model using the combined MDACC and NHS/HPFS dataset; although clinical variables included only age and sex in the combined dataset, the two SNPs together showed a clear potential as a key predictor of 5-y survival for CM. Furthermore, we noticed a sharp rise in predictive

performance of 5-y ROC curves and time-dependent AUC after adding risk alleles of the two significant SNPs to the model for stage I CM patients from the MDACC dataset with clinical variables (age, sex, ulceration, thickness, mitosis rate, and stage) as other classifiers; in particular, this improvement in performance suggested that these two SNPs should be further tested in the future development of prognostic criteria applicable to stage I CM patients, once validated.

To date, however, no published results have linked *SDCCAG8* or *MAGI2* to CM patient survival. *SDCCAG8*, also known as serologically defined colon cancer antigen 8, has been identified as playing an important role in controlling centrosomal properties and recruitment function.³² Although few reports have investigated the roles of *SDCCAG8* in CM, a recent study demonstrated that the down-regulation of *SDCCAG8* significantly suppressed the progression of head and neck squamous cell carcinoma.³³ Additionally, other previous studies also linked variations in the expression of *SDCCAG8* to the invasion of tumors, as well as to specific treatment options for cancer.^{34,35} To the best of our knowledge, this is the first report of an association between *SDCCAG8* and CM patient survival. In the present study, the results showed that rs10803138 G>A was also significantly associated with an increased *SDCCAG8* mRNA expression in both lymphoblastoid cell lines and normal skin tissues. Furthermore, in metastatic CM tissues, *SDCCAG8* mRNA accumulated conspicuously and was associated with a worse CM survival. Consistent with published studies on other cancers, these observations suggest that *SDCCAG8* may play an oncogenic role in CM.

MAGI2, also called membrane-associated guanylate kinase, WW and PDZ domain containing protein 2, belongs to the membrane-associated guanylate kinase (MAGUK) family.³⁶ While *MAGI2* functions as a regular scaffold protein in assembling and anchoring cellular signaling complexes,³⁷ the entire MAGUK family (including *MAGI2*) contains a pivotal domain necessary for mitotic spindle alignment along the cortical polarity axis.³⁸ Evidence from previous studies has shown that *MAGI2* was highly expressed in neurons and podocytes, normally associated with nephritic and neurologic disorders.^{39,40} In the present study, we showed that rs3807694 C>T, as a risk factor for CMSS, was associated with an elevated *MAGI2* mRNA expression in normal sun-exposed skin tissues; in addition, compared with primary CM tissues, *MAGI2* mRNA was expressed at significantly elevated levels in metastatic CM tissues. These findings strongly support a potential oncogenic role of *MAGI2* in CM, a possible molecular mechanism underlying the observed association between *MAGI2* rs3807694 and a poor survival in CM patients. Interestingly, some reports have shown that *MAGI2* functions as a tumor suppressor gene in a range of malignant tumors,⁴¹⁻⁴³ but not in melanoma as evidenced in the present study. Therefore, the exact molecular mechanisms implicated in an association between *MAGI2* expression levels and CM survival require additional in-depth mechanistic studies.

Despite the above-mentioned significant observations, several limitations in the present study should be noted. CM patients whose data were used in the present study were recruited from Caucasian populations; therefore, further validation in different ethnic CM patient cohorts should be conducted. Additionally, compared with the MDACC discovery dataset, the NHS/HPFS validation dataset had fewer participants with fewer variables for further analysis, which could reduce statistical power in validating the effects of tagSNPs. Furthermore, some clinical covariates of the study populations were not available in the NHS/HPFS dataset, limiting the validation of the findings; however, the consistent risk associations across different

datasets after stratified analysis suggested that our results might not be seriously biased by the absence of clinical covariates in the validation population. Given the potential prognostic role of the two SNPs in mitosis-related pathway genes in CMSS, these SNPs may serve either as a new prognostic biomarker for CM patients or as a clinical decision-making indicator for their caregivers, once these findings are validated by additional large studies.

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DISCLOSURE

The authors have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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