


## RESEARCH ARTICLE

**Genetic variants in the metzincin metallopeptidase family genes predict melanoma survival<sup>†</sup>**

**An abbreviated title:** Genetic variants in the metzincin metallopeptidase family genes predict melanoma survival

Yinghui Xu<sup>1,2,3\*</sup>, Yanru Wang<sup>1,2\*</sup>, Hongliang Liu<sup>1,2</sup>, Qiong Shi<sup>4</sup>, Dakai Zhu<sup>5</sup>, Christopher I. Amos<sup>5</sup>, Shenyang Fang<sup>6</sup>, Jeffrey E. Lee<sup>6</sup>, Terry Hyslop<sup>1,7</sup>, Xin Li<sup>8</sup>, Jiali Han<sup>9\*\*</sup>, and Qingyi Wei<sup>1,2\*\*</sup> 

<sup>1</sup> Duke Cancer Institute, Duke University Medical Center, Durham, NC 27710, USA

<sup>2</sup> Department of Medicine, Duke University School of Medicine, Durham, NC 27710, USA

<sup>3</sup> Cancer Center, The First Hospital of Jilin University, Changchun, Jilin 130021, China

<sup>4</sup> Department of Dermatology, Xijing Hospital, Xi'an, Shanxi 710032, China

<sup>5</sup> Department of Biomedical Data Science, Geisel School of Medicine, Dartmouth College, Hanover, NH 03755, USA

<sup>6</sup> Department of Surgical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, USA

<sup>7</sup> Department of Biostatistics and Bioinformatics, Duke University and Duke Clinical Research Institute, Durham, NC 27710, USA

<sup>8</sup> Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA

<sup>9</sup> Department of Epidemiology, Fairbanks School of Public Health, and Melvin and Bren Simon Cancer Center, Indiana University, Indianapolis, IN 46202, USA

<sup>†</sup>This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/mc.22716]

**Additional Supporting Information may be found in the online version of this article.**

**Received 20 March 2017; Accepted 8 August 2017**

**Molecular Carcinogenesis**

**This article is protected by copyright. All rights reserved**

**DOI 10.1002/mc.22716**

This is the author's manuscript of the article published in final edited form as:

Xu, Y., Wang, Y., Liu, H., Shi, Q., Zhu, D., Amos, C. I., Fang, S., Lee, J. E., Hyslop, T., Li, X., Han, J. and Wei, Q. (), Genetic variants in the metzincin metallopeptidase family genes predict melanoma survival. *Mol. Carcinog.*. Accepted Author Manuscript.

<http://dx.doi.org/10.1002/mc.22716>

\*Yinghui Xu and Yanru Wang contributed equally to this work.

\*\*Corresponding authors: Qingyi Wei, M.D., Ph.D., Duke Cancer Institute, Duke University Medical Center and Department of Medicine, Duke School of Medicine, 905 S LaSalle Street, Durham, NC 27710, USA, Tel.: (919) 660-0562, E-mail: qingyi.wei@duke.edu and Jiali Han, PhD, Department of Epidemiology, Fairbanks School of Public Health, and Melvin and Bren Simon Cancer Center, Indiana University, Indianapolis, IN 46202, USA, Tel.: (317) 278-4026, jjalihan@iu.edu.

## Acknowledgments

We would like to thank the John Hopkins University Center for Inherited Disease Research for conducting high-throughput genotyping for this study. We thank the participants and staff of the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. We also thank all the individuals who participated in this project. The authors assume full responsibility for analyses and interpretation of these data. The MD Anderson Study was supported by NIH/NCI R01 CA100264, 2P50CA093459 and R01CA133996 as well as by The University of Texas MD Anderson Cancer Center Various Donors Melanoma and Skin Cancers Priority Program Fund; the Miriam and Jim Mulva Research Fund; the McCarthy Skin Cancer Research Fund and the Marit Peterson Fund for Melanoma Research. The Harvard Study was in part supported by NIH/NCI R01 CA49449, P01 CA87969, UM1 CA186107 and UM1 CA167552. Qingyi Wei was supported by start-up funds from Duke Cancer Institute, Duke University Medical Center, and Qingyi Wei and Terry Hyslop were in part supported by the Duke Cancer Institute as part of the P30 Cancer Center Support Grant (Grant ID: NIH CA014236).

**Abbreviations:** CM, cutaneous melanoma; CMSS, cutaneous melanoma-specific survival; HR, hazards ratio; CI, confidence interval; SEER, Surveillance, Epidemiology, and End Results; UV, ultraviolet; GWAS, genome-wide association studies; SNP, single-nucleotide polymorphisms; MDACC, MD Anderson Cancer Center; NHS, Nurses' Health Study; HPFS, Health Professionals Follow-up Study; MAF, minor allelic frequency; HWE, Hardy-Weinberg equilibrium; FPRP, false-positive report probability; ROC, receiver operating characteristic; AUC, area under the curve; eQTL, expression quantitative trait loci; MMP16, matrix metalloproteinase 16; MMP9, matrix metalloproteinase 9; ADAMTS, a disintegrin-metalloproteinases with thrombospondin domains; ADAMTS3, ADAM metalloproteinase with thrombospondin type 1 motif 3; TLL2, toll-like protein 2.

## ABSTRACT

Metzincins are key molecules in the degradation of the extracellular matrix and play an important role in cellular processes such as cell migration, adhesion, and cell fusion of malignant tumors, including cutaneous melanoma (CM). We hypothesized that genetic variants of the metzincin metallopeptidase family genes would be associated with CM-specific survival (CMSS). To test this hypothesis, we first performed Cox proportional hazards regression analysis to evaluate the associations between genetic variants of 75 metzincin metallopeptidase family genes and CMSS using the dataset from the genome-wide association study (GWAS) from The University of Texas MD Anderson cancer Center (MDACC) which included 858 non-Hispanic white patients with CM, and then validated in the dataset from the Harvard GWAS study which had 409 non-Hispanic white patients with invasive CM. Four independent SNPs (*MMP16* rs10090371 C>A, *ADAMTS3* rs788935 T>C, *TLL2* rs10882807 T>C and *MMP9* rs3918251 A>G) were identified as predictors of CMSS, with a variant-allele attributed hazards ratio (HR) of 1.73 (1.32-2.29, 9.68E-05), 1.46 (1.15-1.85, 0.002), 1.68 (1.31-2.14, 3.32E-05) and 0.67 (0.51-0.87, 0.003), respectively, in the meta-analysis of these two GWAS studies. Combined analysis of risk genotypes of these four SNPs revealed a decreased CMSS in a dose-response manner as the number of risk genotypes increased ( $P_{\text{trend}} < 0.001$ ). An improvement was observed in the prediction model [area under the curve (AUC) = 81.4% to 78.6%], when these risk genotypes were added to the model containing non-genotyping variables. Our findings suggest that these genetic variants may be promising prognostic biomarkers for CMSS. This article is protected by copyright. All rights reserved

**Keywords:** cutaneous melanoma; genome-wide association study (GWAS); single-nucleotide polymorphism (SNP) ; metzincins; cutaneous melanoma-specific survival (CMSS)

## INTRODUCTION

Cutaneous melanoma (CM) is the fifth most common cancer in the United States, and its incidence rate is increasing by 3% annually [1]. Early diagnosis, immunomodulation (e.g., anti-CTLA4) and targeted therapy (e.g., *BRAF* and *MEK* inhibitors) have made breakthrough improvements in prognosis of advanced-stage CM patients [2,3]. The five-year (2006-2012) survival rate of CM is estimated to be about 91.5% based on data from the Surveillance, Epidemiology, and End Results (SEER) program.

CM is a complex disease that originates from melanocytes primarily found in the skin, risk of developing melanoma is influenced by both environmental and host factors. For example, ultraviolet (UV) exposure, an important environmental factor, has been recognized as an independent risk factor for CM [4], which not only increases CM risk but also leads to tumor progression by affecting molecular signaling pathways and inhibiting immune reactions [4]. Host factors such as color of the skin, hair, and eyes, as well as genetic variants, have also been identified to be involved in CM development and progression [4]. In distinction to somatic mutations, germline variants with a low penetrance have a high frequency in the general population. In recent years, large-scale genome-wide association studies (GWASs) have identified a number of genetic variants as risk factors of many complex diseases, including CM [5]. Several single-nucleotide polymorphisms (SNPs) (such as rs7526389, rs1539188, rs1049481 and rs2974755) have been found to be independent predictors of CM prognosis [6]. However, GWASs may have identified many of the most statistically significant SNPs but also may have missed biologically functional and mechanistically important genetic variants that do not rank among the top SNPs. Recently, hypothesis-driven and pathway-based (or gene set-based) approaches have been effectively used to search for novel functional genetic variants that are associated with risk and prognosis of CM [7]. For example, *PIWIL4* rs7933369 and rs508485 and *DCP1A* rs11551405 in the PIWI-piRNA pathway [8] and *VDBP* rs12512631 and *RXRA* rs7850212 in the vitamin D pathway [9] were found to be associated with CM prognosis. Investigations of functional genes and SNPs have provided additional evidence for the biological mechanisms underlying observed associations with CM prognosis [10-12].

Metzincin metallopeptidase family members, including matrixins, adamlysins, astacins, and pappalysins, are calcium-dependent zinc-containing endopeptidases that have proteolytic activities and play an important role in degradation of the extracellular matrix and some protein complexes. It has been

reported that metzincin family genes play an important role in several cancer-progression-related processes, including cell migration, adhesion, and cell fusion of malignant diseases [13-17]. For example, matrix metalloproteinase 9 (*MMP9*) has been reported to be associated with cancer invasiveness and metastasis, and inhibitors against *MMP9* represent a promising strategy for anti-melanoma therapy [18]. *MMP12* expression has been reported to be increased in CM and related to tumor invasion and metastasis [19]. Moreover, high expression of the disintegrin and metalloproteinase domain-containing protein 10 (*ADAM10*) was found to be related to melanoma metastasis [20]. The disintegrin-metalloproteinases with thrombospondin domains (*ADAMTS*) genes have been suggested to act as tumor suppressors in various cancers, including melanoma, and *ADAMTS18* mutations can promote cell growth, migration, and metastasis of melanoma [21]. In addition, other metzincin family members, including astacins and pappalysins, have also been reported to be associated with tumorigenesis [22,23].

To date, there are no reported studies using large-scale GWAS datasets to investigate the role of genetic variants of genes in the metzincin metalloproteinase family in melanoma survival. We hypothesize that genetic variants of the metzincin metalloproteinase family genes would be associated with CM-specific survival (CMSS).

## **MATERIALS AND METHODS**

### **Study populations**

The discovery dataset included 858 non-Hispanic white patients with CM from a previously published GWAS study at The University of Texas MD Anderson Cancer Center (MDACC), who were recruited between March 1993 and August 2008 [24]. The GWAS database of genotypes and phenotypes, including patient age, sex, primary tumor Breslow thickness, metastasis, ulceration, mitotic rate and survival outcome, were available at the dbGaP (accession: phs000187.v1.p1) [25]. In this study, genomic DNA extracted from the blood samples was genotyped with Illumina HumanOmni-Quad\_v1\_0\_B array. Genome-wide imputation (imputation quality  $r^2 \geq 0.8$ ) was conducted with the MACH software based on the 1000 Genomes CEU population (March 2010 release) [26].

The replication dataset included 409 non-Hispanic white patients with invasive CM in the two cohorts of Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) from Harvard

University, from which the information of age, sex, survival outcome and genotype data were available. Genotyping was performed using the Illumina HumanHap610 array. Genome-wide imputation (imputation quality  $r^2 \geq 0.8$ ) was also performed using the MACH software based on the 1000 Genomes Project CEU population (March 2012 release) [27,28].

All individuals in the two datasets participated in these studies after providing a written informed consent under an Institutional Review Board-approved protocol.

### **Gene and SNP extraction**

The metzincin metalloproteinase family genes were selected from the HUGO gene family website (<http://www.genenames.org/cgi-bin/genefamilies/set/901>). Genotyped and imputed SNPs of the metzincin metalloproteinase family genes were selected to be analyzed with the following quality control criteria: (1) a genotyping rate  $\geq 95\%$ , (2) a minor allelic frequency (MAF)  $\geq 0.05$ , and (3) Hardy-Weinberg equilibrium (HWE)  $P$  value  $\geq 1 \times 10^{-5}$ .

### **Statistical analysis**

CMSS was considered the major end-point in the present study, which was defined as the date from the diagnosis of malignant CM to the time of CM-related death or the time of the last follow-up. In the MDACC dataset, Cox proportional hazards regression analysis was performed with adjustment for age, sex, Breslow thickness, metastasis, ulceration and mitotic rate (in an additive genetic model). We estimated the associations between SNPs in the metzincin metalloproteinase family genes and CMSS by calculating hazards ratio (HR) and its 95% confidence interval (CI) using the GenABEL package of R software. In the Harvard dataset, only age and sex were available for adjustment in the further Cox regression analysis. The false-positive report probability (FPRP) method with a cut-off value of 0.20 was used for multiple testing corrections [29]. FPRP was chosen because many imputed SNPs were in linkage disequilibrium (LD) among all the SNPs under investigation, and also it is calculated based on three factors, including the observed  $P$  value, the prior probability of a true association of the tested genetic variant with a disease, and the statistical power of the test. In the present study, we assigned a prior probability of 0.10 to detect an HR of 2.0 for an association with variant genotypes or minor alleles of

the SNPs with  $P \leq 0.05$ . Then, we performed the multivariable stepwise Cox regression analysis including clinical variables and validated SNPs to select the independent representative SNPs in the MDACC dataset, and a meta-analysis was followed to combine the results between the MDACC and Harvard studies using PLINK 1.07. A fixed-effects model was used when no heterogeneity was found between two studies (Q-test  $P$ -value  $> 0.10$  and  $I^2 < 50.0\%$ ); otherwise, a random-effects model was applied. Kaplan-Meier curve and log-rank test were used to estimate the effects of risk genotypes on the cumulative probability of CMSS. Furthermore, we summarized and combined the risk genotypes to assess associations between the number of risk genotypes and CMSS. The heterogeneity test of associations between subgroups of each clinical variable was conducted by using the Chi-square-based Q-test in stratified analyses, and  $P < 0.05$  was considered significant for differences between the subgroups of each clinical variable. A time-dependent receiver operating characteristic (ROC) analysis was performed to calculate area under curve (AUC) of SNPs and clinical variables by using "survAUC" package of R software in the MDACC dataset [30]. In addition, Haploview v4.2 [31] was used to construct a Manhattan plot, and LocusZoom [32] was used to produce regional association plots. All statistical analyses were performed with SAS software (version 9.4; SAS Institute, Cary, NC, USA), if not specified otherwise.

## RESULTS

### Gene and SNP extraction

Seventy-eight metzincin metallopeptidase family genes were selected from the HUGO gene family website (<http://www.genenames.org/cgi-bin/genefamilies/set/901>) (**Supplementary Table 1**). Three pseudogenes (*ADAM1B*, *ADAM24P* and *ADAM3B*) were excluded from the gene list. After quality checks, 13,850 SNPs of 75 genes (i.e., 2,145 genotyped and 11,705 imputed SNPs) were extracted from the imputed MDACC GWAS dataset for further survival analysis.



## Associations between SNPs in the metzincin metallopeptidase family genes and CMSS in the MDACC dataset

We present the workflow of the analyses in **Figure 1**. The basic characteristics of the MDACC and Harvard studies were described previously [24,33] (**Supplementary Table 2**). We first performed Cox regression analysis with adjustment for age, sex, Breslow's thickness, metastasis, ulceration and mitotic rate to evaluate associations between 13,850 SNPs of the metzincin metallopeptidase family genes and CMSS in single locus analysis. Among these SNPs, 570 SNPs were significantly associated with CMSS at  $P \leq 0.05$  in an additive genetic model. We then conducted multiple testing corrections for these 570 SNPs, and 322 SNPs with  $FPRP \leq 0.20$  were selected for validation in another independent dataset of the Harvard study (**Supplementary Figure 1**).

## Replication of the significant SNPs in the Harvard dataset

We validated the 322 SNPs by using the Harvard dataset. After Cox regression analysis with the adjustment for age and sex, eight SNPs remained significantly associated with CMSS at  $P \leq 0.05$  in an additive genetic model, including four SNPs (rs10090371, rs62525943, rs12674820, and rs7013966) in *MMP16*, two SNPs (rs788933 and rs788935) in *ADAMTS3*, one SNP (rs10882807) in *TLL2*, and one SNP (rs3918251) in *MMP9* (**Table 1**).

## Independent representative SNPs

We then performed a stepwise Cox regression analysis of selected clinical variables from the MDACC dataset plus the eight validated SNPs to identify independent predictors of CMSS from the eight validated SNPs (**Table 2**). Four SNPs rs10090371, rs788935, rs10882807 and rs3918251 remained significant in the final model and thus were selected as independent representative SNPs for further analysis. All genotyped and imputed SNPs are shown in the regional association plots with an expansion of 250 KB in the flanks of the gene region, in which

the selected four independent representative SNPs, as shown on the top of the plots, are labeled in purple (**Supplementary Figure 2**).

### **Survival analyses of the four independent SNPs and CMSS in MDACC and Harvard studies**

We performed survival analysis with different genetic models for each independent SNP. As shown in **Table 1**, we found that under an additive genetic model, *MMP16* rs10090371 A, *ADAMTS3* rs788935 C and *TLL2* rs10882807 C variant alleles were associated with an increased death risk of CM, with a variant-allele attributed HR of 1.70 (95% CI = 1.19-2.43,  $P = 0.003$ ), 1.41 (95% CI = 1.05-1.89,  $P = 0.023$ ) and 1.63 (95% CI = 1.19-2.22,  $P = 0.002$ ) in the MDACC study and 1.79 (95% CI = 1.15-2.79,  $P = 0.010$ ), 1.55 (95% CI = 1.03-2.33,  $P = 0.034$ ) and 1.76 (95% CI = 1.18-2.63,  $P = 0.005$ ) in the Harvard study and 1.73 (95% CI = 1.32-2.29,  $P = 9.68E-05$ ), 1.46 (95% CI = 1.15-1.85,  $P = 0.002$ ) and 1.68 (95% CI = 1.31-2.14,  $P = 3.32E-05$ ) in a meta-analysis of the two studies. In addition, the *MMP9* rs3918251 G allele was associated with a decreased death risk of CM, with a variant-allele attributed HR of 0.69 (95% CI = 0.50-0.95,  $P = 0.025$ ) in the MDACC study and 0.63 (95% CI = 0.40-1.00,  $P = 0.050$ ) in the Harvard study and 0.67 (95% CI = 0.51-0.87,  $P = 0.003$ ) in a meta-analysis of the two studies. The univariate and multivariate Cox regression analyses with different genotype models (codominant/dominant/additive) of each representative SNP are presented in **Table 3**.

### **Combined genotype analyses of the four independent representative SNPs**

We combined the risk genotypes of rs10090371 CA+AA, rs788935 TC+CC, rs10882807 TC+CC and rs3918251 AA into a genetic score to assess the joint effect of the four independent SNPs on CMSS. We first combined groups of 0 and 1 risk genotypes into one group, because of their small number of subjects, and categorized all other patients into four groups (i.e., 0 to 4 genetic scores, **Table 3**). Results suggested a risk-genotype dose-response in the effect on

Accepted Article

CMSS associated with the genetic score ( $P_{\text{trend}} < 0.001$  in both MDACC and Harvard studies) after adjustments (**Table 3**). We further dichotomized the patients into a low-score risk group (0-2 risk genotypes) and a high-score risk group (3-4 risk genotypes). A similar result was observed that the high-score risk group had an increased risk of death with an HR of 3.55 (95% CI = 2.30-5.50,  $P < 0.001$ ) in the MDACC study and an HR of 2.77 (95% CI = 1.56-4.90,  $P < 0.001$ ) in the Harvard study, compared with the low-score risk group. Kaplan-Meier curves were also provided to illustrate the association between the number of risk genotypes and CMSS (**Figure 2A-D**).

### **Stratified analyses for the effect of combined risk genotypes on CMSS**

We then conducted stratified analyses to evaluate whether the combined effect of risk genotypes as defined by the genetic score on CMSS was modified by clinical characteristics, including age, sex, metastasis, Breslow thickness, ulceration and mitotic rate in the MDACC dataset and age and sex in the Harvard dataset. In the MDACC dataset, we found that a high-score risk genotypes was associated with an increased risk of CM death with HR of 2.19 in the non-metastasis group, and 6.38 in the regional or distant metastasis group, and heterogeneity was observed between these two subgroups ( $P = 0.018$ ) (**Supplementary Table 3**). No heterogeneity was found in the subgroups of the Harvard dataset.

### **ROC curve and time-dependent AUC estimators in the MDACC study**

We used the estimates for the ROC curve and the time-dependent AUC in the MDACC study to assess the improvement in prediction accuracy when including the four independent SNPs in the presence of other host and clinical variables (i.e., age, sex, metastasis, Breslow thickness, ulceration and mitotic rate). From the ROC curve, we found that the combination of clinical variables and risk genotypes enhanced the prediction effect of five-year CMSS, compared with the group of clinical variables only (AUC = 81.4% to 78.6%), and the time-

dependent AUC curve showed this effect from the beginning to the end of the follow-up time (Figure 2E-F). We did not evaluate ROC curve and time-dependent AUC estimators in the Harvard dataset, because clinical variables other than age and sex were unavailable.

### eQTL analyses

We further analyzed the associations between the four independent SNPs and levels of the corresponding gene mRNA expression (i.e., expression quantitative trait loci analysis, eQTL analysis) using the data from the GTEx Portal (<http://www.gtexportal.org/home/>), which has the data for *MMP16* rs10090371 in thyroid tissue, its moderate LD SNP rs12674820 in *MMP16* ( $r^2 = 0.44$ ) in adipose (subcutaneous) tissue and *TLL2* rs10882807 in skin tissue. As shown in **Supplementary Figure 3A-C**, *MMP16* rs10090371 A, *MMP16* rs12674820 G and *TLL2* rs10882807 C alleles were associated with an increase in the corresponding gene mRNA expression levels with *P* values of 2.00E-05, 6.50E-10 and 1.30E-07, respectively. Because there were no expression data for the other two SNPs (*ADAMTS3* rs788935 and *MMP9* rs3918251) in the GTEx Portal, we further explored the potential function for these two SNPs by using the ENCODE project data. As shown in **Supplementary Figure 3D-E**, *ADAMTS3* rs788935 is located at the intron region that shows H3K4Me1 enrichment, and *MMP9* rs3918251 is also located at the intron region that is a DNase I hypersensitive area.

### DISCUSSION

Metzincins are considered key molecules in degradation of the extracellular matrix and play an important role in a variety of biological processes and pathological disorders, such as asthma, rheumatoid arthritis and cancer [34], including CM. The alterations of the metzincin metallopeptidase family genes in CM development and progression have been previously reported [18-21].

In the present study, we performed survival analysis for genetic variants in 75 metzincin metallopeptidase family genes and CMSS using the available MDACC and Harvard GWAS datasets. Four independent representative SNPs (*MMP16* rs10090371 C>A, *ADAMTS3* rs788935 T>C, *TLL2* rs10882807 T>C and *MMP9* rs3918251 A>G) were identified as predictors of CMSS. Specifically, rs10090371A, rs788935C and rs10882807C alleles were associated with a poor CMSS, and the rs3918251G allele was associated with a favorable outcome of CM. When we considered these four risk genotypes together, we also found that there was a risk-genotype dose-response in the effect on CMSS associated with the genetic score combining the four risk genotypes (rs10090371 CA+AA, rs788935 TC+CC, rs10882807 TC+CC and rs3918251 AA). These four independent SNPs highlighted the roles of four genes (*MMP16*, *ADAMTS3*, *TLL2* and *MMP9*) in CM patient survival.

*MMP16*, located at 8q21.3, encodes an enzyme called matrix metalloproteinase 16, which is a family member of matrix metalloproteinases (MMPs). Like the other MMPs, *MMP16* is also associated with cancer cell proliferation, invasion and metastasis [35]. It is suggested that *MMP16* contributes to a poor prognosis in gastric cancer by promoting tumor cell proliferation and invasion [35]. Other studies have reported that *MMP16* was associated with the migration and invasion of glioma and pancreatic cancer [36,37]. Therefore, targeting *MMP16* may be a feasible approach for inhibiting the progression of several cancers. Furthermore, *MMP16* has been proposed to influence cell-cell adhesion and lymphatic invasion in melanoma [38]. Taken together, *MMP16* may be considered to act as an oncogene and contribute to poor prognosis in multiple cancers, including CM. In the present study, the rs10090371 AA variant genotype was associated with an decreased CMSS, compared with the CC genotype, and the AA genotype was also associated with an increased *MMP16* mRNA expression in thyroid tissue, although we did not have the data for skin or cutaneous tissue from the GTEx portal, another SNP rs12674820 in moderate LD ( $r^2 = 0.44$ ) with rs10090371 was associated with increased *MMP16* mRNA expression in subcutaneous tissue, which was consistent with the result of rs10090371

in thyroid tissue. Therefore, it appears likely that SNPs in this region may influence gene function by mediating mRNA expression levels in multiple tissue types.

*ADAMTS3*, located at 4q13.3, encodes an enzyme called ADAM metalloproteinase with thrombospondin type 1 motif 3. As one of the adamalysins family genes, *ADAMTS3* also participates in various cellular processes, including extracellular matrix degradation, cleavage of proteoglycans, inhibition of angiogenesis, gonadal development and organogenesis [39]. One study reported that *ADAMTS3* was downregulated in breast cancer [39]. To date, there is no report about the role of *ADAMTS3* in melanoma. In the present study, we found an association between the *ADAMTS3* rs788935 and CM prognosis. According to the ENCODE project data from UCSC, rs788935 is located at the intron region of *ADAMTS3*, which demonstrates considerable levels of H3K4Me1 enrichment that is accessible to transcription factors to enhance transcriptional activity. Therefore, it appears likely that SNPs in this region may influence gene expression by mediating the transcriptional activity.

*TLL2*, located at 10q24.1, encodes a protein called tolloid-like protein 2, which is an astacin-like zinc-dependent metalloprotease and is a subfamily member of the metzincin family. In the present study, this is the first report of an association between the *TLL2* rs10882807CC variant genotype and CM survival, and likely this genotype increases *TLL2* mRNA expression in a variant allele dose-response manner in skin tissue. Therefore, we propose that *TLL2* may function as an oncogene to influence the melanoma progression. We acknowledge that additional functional studies are needed to validate our findings.

*MMP9*, located at 20q13.12, encodes an enzyme called matrix metalloproteinase 9 that also belongs to the MMPs family. *MMP9* has been reported to be associated with the development and progression of many cancers. For example, one study reported that upregulating of *MMP9* expression promoted hepatocellular carcinoma cell migration and invasion [40]. Another study suggested that increased *MMP9* expression was associated with gastric cancer cell invasion [41]. In addition, *MMP9* activity has been reported to be correlated

with prognosis of other cancers, including cancers of the lung [42], colorectum [43], esophagus [44] and breast [45]. Importantly, transcript levels of *MMP9* were also observed to be increased in melanoma tumors, compared with that of melanocyte controls [46]. Additionally, it has been reported that *MMP9* silencing inhibited mouse melanoma cell invasion and migration both *in vitro* and *in vivo*, suggesting that *MMP9* might have promising applications for target therapy of CM [47]. Taken together, these data suggest that *MMP9* acts as an oncogene contributing to poor prognosis across multiple cancers, including CM. In the present study, we found that the rs3918251 GG variant genotype was a protective factor for CMSS. According to the ENCODE project data from UCSC, rs3918251 is located at the DNase I hypersensitive area, where has lost the condensed structure, exposing the DNA and making it accessible to DNase I and transcription factors, plausibly influencing transcriptional activity. However, stronger functional evidence is needed to unravel the biological mechanisms underlying the observed association with CM survival.

There are some limitations in the present study. First, the MDACC study included clinical variables such as age, sex, primary tumor Breslow thickness, regional/distant metastasis, ulceration and mitotic rate for adjustment, but the Harvard study included only age and sex. Furthermore, additional potentially important clinical variables were not available for inclusion, such as performance status, nutritional status, tumor somatic mutation data, and details regarding treatment and response. Second, the study patients by design were all non-Hispanic whites, therefore, our findings cannot be generalized to the general populations, and validations in other ethnic groups are needed. Third, the GTEx portal and other biological function prediction websites are limited in their ability to definitively evaluate the function of the SNPs identified. More functional evidence is needed, and potential biological mechanisms should be explored by using the accessible melanoma tissues.

In conclusion, we evaluated associations between genetic variants of 75 metzincin metallopeptidase family genes and CMSS using MDACC and Harvard GWAS datasets. We identified *MMP16* rs10090371 C>A, *ADAMTS3* rs788935 T>C, *TLL2* rs10882807 T>C and *MMP9* rs3918251 A>G as possible predictors for CMSS. Additional population replications from other ethnic groups and functional validation from mechanistic studies are needed to further validate our results. Once validated, our findings may provide promising prognostic biomarkers for personalized management and treatment of CM patients.

#### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.



## REFERENCES

1. Tripp MK, Watson M, Balk SJ, Swetter SM, Gershenwald JE. State of the science on prevention and screening to reduce melanoma incidence and mortality: The time is now. *CA Cancer J Clin* 2016.
2. Eggermont AM, Spatz A, Robert C. Cutaneous melanoma. *Lancet* 2014;383(9919):816-827.
3. Flaherty KT, Hodi FS, Fisher DE. From genes to drugs: targeted strategies for melanoma. *Nat Rev Cancer* 2012;12(5):349-361.
4. Pejnova S, Dzokic G, Tudzarova-Gjorgova S, Panov S. Molecular Biology and Genetic Mechanisms in the Progression of the Malignant Skin Melanoma. *Pril (Makedon Akad Nauk Umet Odd Med Nauki)* 2016;37(2-3):89-97.
5. Vaysse A, Fang S, Brossard M, Wei Q, Chen WV, Mohamdi H, Vincent-Fetita L, Margaritte-Jeannin P, Lavielle N, Maubec E, Lathrop M, Avril MF, Amos CI, Lee JE, Demenais F. A comprehensive genome-wide analysis of melanoma Breslow thickness identifies interaction between CDC42 and SCIN genetic variants. *Int J Cancer* 2016;139(9):2012-2020.
6. Fang S, Vaysse A, Brossard M, Wang Y, Deng D, Liu Q, Zhang P, Xu K, Li M, Feng R, Liu H, Dang Y, Chen W, Prieto V, Gershenwald JE, Ross MI, Matejka B, Malke J, Haydu LE, Reveille JD, Sui D, Bassett RL, Jr., Koshkina N, Avril MF, Lu M, Wei Q, Demenais F, Amos CI, Lee JE. Melanoma Expression Genes Identified through Genome-Wide Association Study of Breslow Tumor Thickness. *J Invest Dermatol* 2016.
7. Wang K, Li M, Bucan M. Pathway-based approaches for analysis of genomewide association studies. *Am J Hum Genet* 2007;81(6):1278-1283.
8. Zhang W, Liu H, Yin J, Wu W, Zhu D, Amos CI, Fang S, Lee JE, Li Y, Han J, Wei Q. Genetic variants in the PIWI-piRNA pathway gene DCP1A predict melanoma disease-specific survival. *Int J Cancer* 2016;139(12):2730-2737.
9. Yin J, Liu H, Yi X, Wu W, Amos CI, Fang S, Lee JE, Han J, Wei Q. Genetic variants in the vitamin D pathway genes VDBP and RXRA modulate cutaneous melanoma disease-specific survival. *Pigment Cell Melanoma Res* 2016;29(2):176-185.
10. Yuan H, Liu H, Liu Z, Zhu D, Amos CI, Fang S, Lee JE, Wei Q. Genetic variants in Hippo pathway genes YAP1, TEAD1 and TEAD4 are associated with melanoma-specific survival. *Int J Cancer* 2015;137(3):638-645.
11. Park JY, Amankwah EK, Anic GM, Lin HY, Walls B, Park H, Krebs K, Madden M, Maddox K, Marzban S, Fang S, Chen W, Lee JE, Wei Q, Amos CI, Messina JL, Sondak VK, Sellers TA, Egan KM. Gene variants in angiogenesis and lymphangiogenesis and cutaneous melanoma progression. *Cancer Epidemiol Biomarkers Prev* 2013;22(5):827-834.
12. Li C, Yin M, Wang LE, Amos CI, Zhu D, Lee JE, Gershenwald JE, Grimm EA, Wei Q. Polymorphisms of nucleotide excision repair genes predict melanoma survival. *J Invest Dermatol* 2013;133(7):1813-1821.
13. Walkiewicz K, Getek M, Muc-Wierzgon M, Kokot T, Nowakowska-Zajdel E. [The importance of ADAM family proteins in malignant tumors]. *Postepy Hig Med Dosw (Online)* 2016;70:67-73.
14. Binder MJ, McCoombe S, Williams ED, McCulloch DR, Ward AC. The extracellular matrix in cancer progression: Role of hyaluronan proteoglycans and ADAMTS enzymes. *Cancer Lett* 2016;385:55-64.
15. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014;15(12):786-801.
16. Thakur V, Bedogni B. The membrane tethered matrix metalloproteinase MT1-MMP at the forefront of melanoma cell invasion and metastasis. *Pharmacol Res* 2016;111:17-22.

17. Moro N, Mauch C, Zigrino P. Metalloproteinases in melanoma. *Eur J Cell Biol* 2014;93(1-2):23-29.
18. Medeiros Turra K, Pineda Rivelli D, Berlanga de Moraes Barros S, Mesquita Pasqualoto KF. Constructing and Validating 3D-pharmacophore Models to a Set of MMP-9 Inhibitors for Designing Novel Anti-melanoma Agents. *Mol Inform* 2016;35(6-7):238-252.
19. Zhang Z, Zhu S, Yang Y, Ma X, Guo S. Matrix metalloproteinase-12 expression is increased in cutaneous melanoma and associated with tumor aggressiveness. *Tumour Biol* 2015;36(11):8593-8600.
20. Caltabiano R, Puzzo L, Barresi V, Ieni A, Loreto C, Musumeci G, Castrogiovanni P, Ragusa M, Foti P, Russo A, Longo A, Reibaldi M. ADAM 10 expression in primary uveal melanoma as prognostic factor for risk of metastasis. *Pathol Res Pract* 2016;212(11):980-987.
21. Wei X, Prickett TD, Vilorio CG, Molinolo A, Lin JC, Cardenas-Navia I, Cruz P, Program NCS, Rosenberg SA, Davies MA, Gershenwald JE, Lopez-Otin C, Samuels Y. Mutational and functional analysis reveals ADAMTS18 metalloproteinase as a novel driver in melanoma. *Mol Cancer Res* 2010;8(11):1513-1525.
22. Lottaz D, Maurer CA, Noel A, Blacher S, Huguenin M, Nievergelt A, Niggli V, Kern A, Muller S, Seibold F, Friess H, Becker-Pauly C, Stocker W, Sterchi EE. Enhanced activity of meprin-alpha, a pro-migratory and pro-angiogenic protease, in colorectal cancer. *PLoS One* 2011;6(11):e26450.
23. Takabatake Y, Oxvig C, Nagi C, Adelson K, Jaffer S, Schmidt H, Keely PJ, Eliceiri KW, Mandeli J, Germain D. Lactation opposes pappalysin-1-driven pregnancy-associated breast cancer. *EMBO Mol Med* 2016;8(4):388-406.
24. Amos CI, Wang LE, Lee JE, Gershenwald JE, Chen WV, Fang S, Kosoy R, Zhang M, Qureshi AA, Vattathil S, Schacherer CW, Gardner JM, Wang Y, Bishop DT, Barrett JH, Geno MELI, MacGregor S, Hayward NK, Martin NG, Duffy DL, Investigators QM, Mann GJ, Cust A, Hopper J, Investigators A, Brown KM, Grimm EA, Xu Y, Han Y, Jing K, McHugh C, Laurie CC, Doheny KF, Pugh EW, Seldin MF, Han J, Wei Q. Genome-wide association study identifies novel loci predisposing to cutaneous melanoma. *Hum Mol Genet* 2011;20(24):5012-5023.
25. Mailman MD, Feolo M, Jin Y, Kimura M, Tryka K, Bagoutdinov R, Hao L, Kiang A, Paschall J, Phan L, Popova N, Pretel S, Ziyabari L, Lee M, Shao Y, Wang ZY, Sirotkin K, Ward M, Kholodov M, Zbicz K, Beck J, Kimelman M, Shevelev S, Preuss D, Yaschenko E, Graeff A, Ostell J, Sherry ST. The NCBI dbGaP database of genotypes and phenotypes. *Nat Genet* 2007;39(10):1181-1186.
26. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: Using Sequence and Genotype Data to Estimate Haplotypes and Unobserved Genotypes. *Genetic Epidemiology* 2010;34(8):816-834.
27. Lappalainen T, Sammeth M, Friedlander MR, Hoen PA, Monlong J, Rivas MA, Gonzalez-Porta M, Kurbatova N, Griebel T, Ferreira PG, Barann M, Wieland T, Greger L, van Iterson M, Almlöf J, Ribeca P, Pulyakhina I, Esser D, Giger T, Tikhonov A, Sultan M, Bertier G, MacArthur DG, Lek M, Lizano E, Buermans HP, Padioleau I, Schwarzmayr T, Karlberg O, Ongen H, Kilpinen H, Beltran S, Gut M, Kahlem K, Amstislavskiy V, Stegle O, Pirinen M, Montgomery SB, Donnelly P, McCarthy MI, Flicek P, Strom TM, Geuvadis C, Lehrach H, Schreiber S, Sudbrak R, Carracedo A, Antonarakis SE, Hasler R, Syvanen AC, van Ommen GJ, Brazma A, Meitinger T, Rosenstiel P, Guigo R, Gut IG, Estivill X, Dermitzakis ET. Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* 2013;501(7468):506-511.
28. Biernacka JM, Tang R, Li J, McDonnell SK, Rabe KG, Sinnwell JP, Rider DN, de Andrade M, Goode EL, Fridley BL. Assessment of genotype imputation methods. *BMC Proc* 2009;3 Suppl 7:S5.
29. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst* 2004;96(6):434-442.

30. Chambless LE, Diao G. Estimation of time-dependent area under the ROC curve for long-term risk prediction. *Stat Med* 2006;25(20):3474-3486.
31. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21(2):263-265.
32. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 2010;26(18):2336-2337.
33. Li H, Wang Y, Liu H, Shi Q, Xu Y, Wu W, Zhu D, Amos CI, Fang S, Lee JE, Han J, Wei Q. Genetic variants in the integrin signaling pathway genes predict cutaneous melanoma survival. *Int J Cancer* 2016.
34. Sterchi EE. Special issue: metzincin metalloproteinases. *Mol Aspects Med* 2008;29(5):255-257.
35. Cao L, Chen C, Zhu H, Gu X, Deng D, Tian X, Liu J, Xiao Q. MMP16 is a marker of poor prognosis in gastric cancer promoting proliferation and invasion. *Oncotarget* 2016.
36. Li Y, Wang Y, Yu L, Sun C, Cheng D, Yu S, Wang Q, Yan Y, Kang C, Jin S, An T, Shi C, Xu J, Wei C, Liu J, Sun J, Wen Y, Zhao S, Kong Y. miR-146b-5p inhibits glioma migration and invasion by targeting MMP16. *Cancer Lett* 2013;339(2):260-269.
37. Lin F, Wang X, Jie Z, Hong X, Li X, Wang M, Yu Y. Inhibitory effects of miR-146b-5p on cell migration and invasion of pancreatic cancer by targeting MMP16. *J Huazhong Univ Sci Technolog Med Sci* 2011;31(4):509-514.
38. Tatti O, Gucciardo E, Pekkonen P, Holopainen T, Louhimo R, Repo P, Maliniemi P, Lohi J, Rantanen V, Hautaniemi S, Alitalo K, Ranki A, Ojala PM, Keski-Oja J, Lehti K. MMP16 Mediates a Proteolytic Switch to Promote Cell-Cell Adhesion, Collagen Alignment, and Lymphatic Invasion in Melanoma. *Cancer Res* 2015;75(10):2083-2094.
39. Porter S, Scott SD, Sassoon EM, Williams MR, Jones JL, Girling AC, Ball RY, Edwards DR. Dysregulated expression of adamalysin-thrombospondin genes in human breast carcinoma. *Clin Cancer Res* 2004;10(7):2429-2440.
40. Wang Q, Yu W, Huang T, Zhu Y, Huang C. RUNX2 promotes hepatocellular carcinoma cell migration and invasion by upregulating MMP9 expression. *Oncol Rep* 2016;36(5):2777-2784.
41. Chen SW, Zhang Q, Xu ZF, Wang HP, Shi Y, Xu F, Zhang WJ, Wang P, Li Y. HOXC6 promotes gastric cancer cell invasion by upregulating the expression of MMP9. *Mol Med Rep* 2016;14(4):3261-3268.
42. Yu Y, Ding Z, Jian H, Shen L, Zhu L, Lu S. Prognostic value of MMP9 activity level in resected stage I B lung adenocarcinoma. *Cancer Med* 2016;5(9):2323-2331.
43. Xiao B, Chen D, Luo S, Hao W, Jing F, Liu T, Wang S, Geng Y, Li L, Xu W, Zhang Y, Liao X, Zuo D, Wu Y, Li M, Ma Q. Extracellular translationally controlled tumor protein promotes colorectal cancer invasion and metastasis through Cdc42/JNK/ MMP9 signaling. *Oncotarget* 2016.
44. Klimczak-Bitner AA, Kordek R, Bitner J, Musial J, Szemraj J. Expression of MMP9, SERPINE1 and miR-134 as prognostic factors in esophageal cancer. *Oncol Lett* 2016;12(5):4133-4138.
45. Liang P, Song Z, Chen D, Linghu R, Wang Y, Zhang X, Kou X, Yang J, Jiao S. GINS2 regulates matrix metalloproteinase 9 expression and cancer stem cell property in human triple negative Breast cancer. *Biomed Pharmacother* 2016.
46. Falzone L, Salemi R, Travali S, Scalisi A, McCubrey JA, Candido S, Libra M. MMP-9 overexpression is associated with intragenic hypermethylation of MMP9 gene in melanoma. *Aging (Albany NY)* 2016;8(5):933-944.
47. Tang ZY, Liu Y, Liu LX, Ding XY, Zhang H, Fang LQ. RNAi-mediated MMP-9 silencing inhibits mouse melanoma cell invasion and migration in vitro and in vivo. *Cell Biol Int* 2013;37(8):849-854.

## Figure legends

**Figure 1.** Research flowchart. SNP, single nucleotide polymorphism; CMSS, cutaneous melanoma-specific survival; FPRP, false-positive report probability; ROC, receiver operating characteristic; GWAS, genome-wide association study.

**Figure 2. A-D:** Kaplan-Meier survival curves for melanoma patients of combined analysis of four risk genotypes in *MMP16*, *ADAMTS3*, *TLL2*, and *MMP9* in MDACC and Harvard studies. **A.** Combined analysis of risk genotypes (four groups) in MDACC study; **B.** Combined analysis of risk genotypes (two groups) in MDACC study; **C.** Combined analysis of risk genotypes (four groups) in Harvard study; **D.** Combined analysis of risk genotypes (two groups) in Harvard study. **E-F:** Receiver operating characteristic (ROC) curve and time-dependent area under the ROC curve (AUC) estimation for prediction of melanoma-specific survival using MDACC dataset. **E.** Ten-year melanoma-specific survival rate; **F.** Time-dependent AUC estimation, based on age, sex, Breslow thickness, regional/distant metastasis, ulceration, mitotic rate and the risk genotypes of the four genes.

**Supplementary Figure 1.** Manhattan plot of 13,850 SNPs of metzincin metalloproteinase family genes in the MDACC study. The statistical values across the autosomes for associations between 13,850 SNPs and melanoma-specific survival are plotted as  $-\log_{10} P$  values. The red horizontal line indicates  $P = 0.05$ . The eight validated SNPs are marked with red points.

**Supplementary Figure 2.** Regional association plots of the four independent SNPs in the metzincin metalloproteinase family genes. The left-hand Y-axis shows the  $-\log_{10}$  transformation of  $P$ -value of individual SNPs, which is plotted against the chromosomal base-pair position with an extension of 250 KB in flanking region of the genes. The right-hand Y-axis shows the recombination rate estimated for European population from HapMap Data Rel 22/phase II. **A.** *MMP16* rs10090371; **B.** *ADAMTS3* rs788935; **C.** *TLL2* rs10882807; **D.** *MMP9* rs3918251.

**Supplementary Figure 3. A-C:** Tissue-specific eQTL analyses of the SNPs (rs10090371, rs12674820 and rs10882807) and corresponding gene mRNA expression. All the data are from oncomine website (<https://www.oncomine.org/resource/main.html>). **A.** *MMP16* rs10090371 ( $P = 2.00E-05$ ); **B.** *MMP16* rs12674820 ( $P = 6.50E-10$ ); **C.** *TLL2* rs10882807 ( $P = 1.30E-07$ ) (There are no tissue-specific eQTL results about *ADAMTS3* rs788935 and *MMP9* rs3918251). **D-E:** Annotations of rs788935 and rs3918251 in the metzincin metallopeptidase family genes from ENCODE project data on the University of California Santa Cruz (UCSC, <http://genome.ucsc.edu/>). **D.** *ADAMTS3* rs788935; **E.** *MMP9* rs3918251.

**Table 1.** Meta-analysis of eight validated SNPs using two published melanoma GWAS datasets

SNP	Allele <sup>a</sup>	Gene	MDACC (n=858)			Harvard (n=409)			Meta-analysis				Prior probability <sup>f</sup>				
			EAF	HR (95%CI) <sup>b</sup>	P <sup>b</sup>	EAF	HR (95%CI) <sup>c</sup>	P <sup>c</sup>	P <sub>het</sub> <sup>d</sup>	I <sup>2</sup>	HR (95%CI) <sup>e</sup>	P <sup>e</sup>	0.25	0.1	0.01	0.001	0.0001
rs10090371	C/A	<i>MMP16</i>	0.20	1.70 (1.19-2.43)	0.003	0.18	1.79 (1.15-2.79)	0.010	0.86	0	1.73 (1.32-2.29)	9.68E-05	0.012	0.036	0.291	0.805	0.976
rs62525943	C/T	<i>MMP16</i>	0.20	1.70 (1.19-2.43)	0.003	0.18	1.74 (1.11-2.74)	0.016	0.94	0	1.72 (1.30-2.27)	1.52E-04	0.012	0.036	0.291	0.805	0.976
rs12674820	C/G	<i>MMP16</i>	0.31	1.47 (1.07-2.03)	0.017	0.28	1.81 (1.19-2.74)	0.005	0.44	0	1.59 (1.23-2.05)	3.42E-04	0.051	0.138	0.638	0.947	0.994
rs7013966	C/T	<i>MMP16</i>	0.43	1.42 (1.04-1.92)	0.027	0.40	1.53 (1.01-2.31)	0.045	0.78	0	1.46 (1.14-1.87)	0.003	0.076	0.198	0.731	0.965	0.996
rs788933	G/A	<i>ADAMTS3</i>	0.42	1.41 (1.05-1.89)	0.023	0.42	1.55 (1.03-2.33)	0.034	0.71	0	1.46 (1.15-1.85)	0.002	0.065	0.173	0.696	0.959	0.996
rs788935	T/C	<i>ADAMTS3</i>	0.42	1.41 (1.05-1.89)	0.023	0.42	1.55 (1.03-2.33)	0.034	0.71	0	1.46 (1.15-1.85)	0.002	0.065	0.173	0.696	0.959	0.996
rs10882807	T/C	<i>TLL2</i>	0.45	1.63 (1.19-2.22)	0.002	0.48	1.76 (1.18-2.63)	0.005	0.77	0	1.68 (1.31-2.14)	3.32E-05	0.007	0.020	0.184	0.694	0.958
rs3918251	A/G	<i>MMP9</i>	0.37	0.69 (0.50-0.95)	0.025	0.36	0.63 (0.40-1.00)	0.050	0.75	0	0.67 (0.51-0.87)	0.003	0.070	0.185	0.714	0.962	0.996

SNP, single nucleotide polymorphisms; GWAS, genome-wide association study; MDACC, The University of Texas MD Anderson cancer center; EAF, effect allele frequency; HR, hazards ratio; CI, confidence interval;

<sup>a</sup>Reference allele/effect allele;

<sup>b</sup>Adjusted for age, sex, Breslow thickness, distant/regional metastasis, ulceration and mitotic rate in Cox models of SNPs and melanoma-specific survival in MDACC study and all the tests of the proportional hazards assumption for the validated SNPs were not significant ( $P > 0.05$ );

<sup>c</sup>Adjusted for age and sex in Harvard study;

<sup>d</sup> $P_{het}$ :  $P$  value for heterogeneity by Cochran's Q test;

**Table 2.** Independent predictors of CMSS as obtained from the stepwise Cox regression analysis of selected variables from the MDACC dataset

Parameter <sup>a</sup>	Category <sup>b</sup>	Frequency	HR (95% CI)	P
Age	≤50/>50	371/487	1.02 (1.01-1.04)	0.010
sex	Female/Male	362/496	1.29 (0.80-2.07)	0.292
Regional/distant metastasis	No/Yes	709/149	4.43 (2.87-6.84)	<0.001
Breslow thickness(mm)	≤1/>1	347/511	1.21 (1.15-1.28)	<0.001
Ulceration	No/Yes	681/155	2.82 (1.83-4.34)	<0.001
Mitotic rate (mm <sup>2</sup> )	≤1/>1	275/583	2.40 (1.17-4.94)	0.017
rs10090371 C>A	CC/CA/AA	546/287/25	1.80 (1.26-2.57)	0.001
rs788935 T>C	TT/TC/CC	277/434/147	1.53 (1.13-2.06)	0.006
rs10882807 T>C	TT/TC/CC	266/418/174	1.63 (1.20-2.22)	0.002
rs3918251 A>G	AA/AG/GG	342/394/122	0.67 (0.49-0.92)	0.015

CMSS, cutaneous melanoma-specific survival; MDACC, The University of Texas MD Anderson cancer Center; HR, hazards ratio; CI, confidence interval;

<sup>a</sup>Stepwise analysis included age, sex, regional/distant metastasis, Breslow thickness, ulceration, mitotic rate and eight SNPs in four genes (rs10090371, rs62525943, rs12674820 and rs7013966 in *MMP16*; rs788933 and rs788935 in *ADAMTS3*; rs10882807 in *TLL2*; and rs3918251 in *MMP9*);

<sup>b</sup>The “category/” was used as the reference.

**Table 3.** Associations between four independent SNPs in the metzincin metallopeptidase family genes and CMSS of patients in the MDACC study and Harvard study

Genotype	MDACC						Harvard					
	Frequency		Univariate analysis		Multivariate analysis <sup>a</sup>		Frequency		Univariate analysis		Multivariate analysis <sup>b</sup>	
	All	Death (%)	HR (95%CI)	P	HR (95%CI)	P	All	Death (%)	HR (95%CI)	P	HR (95%CI)	P
<b><i>MMP16</i> rs10090371 C&gt;A</b>												
CC	546	51 (9.3)	1.00		1.00		277	26 (9.4)	1.00		1.00	
CA	287	39 (13.6)	1.49 (0.98-2.26)	0.062	1.62 (1.05-2.49)	0.028	117	18 (15.4)	1.69 (0.93-3.09)	0.086	1.77 (0.97-3.24)	0.062
AA	25	5 (20.0)	2.13 (0.85-5.33)	0.108	3.30 (1.29-8.43)	0.013	15	4 (26.7)	2.89 (1.01-8.28)	0.048	3.26 (1.13-9.38)	0.029
CA+AA	312	44 (14.1)	1.54 (1.03-2.30)	0.036	1.72 (1.13-2.61)	0.011	132	22 (16.7)	1.83 (1.04-3.23)	0.037	1.93 (1.09-3.41)	0.023
Trend				0.024		0.003				0.018		0.010
<b><i>ADAMTS3</i> rs788935 T&gt;C</b>												
TT	277	22 (7.9)	1.00		1.00		135	11 (8.2)	1.00		1.00	
TC	434	55 (12.7)	1.65 (1.01-2.71)	0.046	1.99 (1.19-3.31)	0.008	202	24 (11.9)	1.46 (0.71-2.98)	0.301	1.48 (0.72-3.02)	0.285
CC	147	18 (12.2)	1.64 (0.88-3.05)	0.121	1.91 (1.00-3.66)	0.052	72	13 (18.1)	2.33 (1.04-5.20)	0.039	2.40 (1.07-5.37)	0.033
TC+CC	581	73 (12.6)	1.65 (1.02-2.66)	0.040	1.97 (1.20-3.22)	0.007	274	37 (13.5)	1.68 (0.86-3.29)	0.132	1.71 (0.87-3.35)	0.120
Trend				0.079		0.023				0.040		0.034
<b><i>TLL2</i> rs10882807 T&gt;C</b>												
TT	266	23 (8.7)	1.00		1.00		120	10 (8.3)	1.00		1.00	
TC	418	53 (12.7)	1.51 (0.93-2.46)	0.099	2.07 (1.22-3.53)	0.007	184	16 (8.7)	1.05 (0.48-2.32)	0.903	1.03 (0.47-2.28)	0.940
CC	174	19 (10.9)	1.30 (0.71-2.39)	0.394	2.62 (1.36-5.06)	0.004	105	22 (21.0)	2.57 (1.22-5.43)	0.013	2.65 (1.25-5.60)	0.011
TC+CC	592	72 (12.2)	1.45 (0.91-2.32)	0.122	2.19 (1.31-3.66)	0.003	289	38 (13.2)	1.60 (0.80-3.21)	0.188	1.60 (0.80-3.21)	0.188
Trend				0.312		0.002				0.007		0.005
<b><i>MMP9</i> rs3918251 A&gt;G</b>												



AA	342	48 (14.0)	1.00		1.00		167	28 (16.8)	1.00		1.00	
AG	394	36 (9.1)	0.61 (0.40-0.94)	0.027	0.52 (0.33-0.82)	0.005	192	14 (7.3)	0.39 (0.21-0.75)	0.004	0.42 (0.22-0.79)	0.008
GG	122	11 (9.0)	0.67 (0.35-1.29)	0.226	0.64 (0.33-1.25)	0.191	50	6 (12.0)	0.66 (0.27-1.58)	0.348	0.64 (0.27-1.56)	0.327
AG+GG	516	47 (9.1)	0.63 (0.42-0.93)	0.022	0.55 (0.36-0.83)	0.004	242	20 (8.3)	0.45 (0.25-0.79)	0.006	0.47 (0.26-0.83)	0.010
Trend				0.055		0.025				0.040		0.050
<b>Number of risk genotypes<sup>c</sup></b>												
0	33	2 (6.1)					10	0 (0.0)				
1	187	13 (7.0)	1.00		1.00		94	4 (4.3)	1.00		1.00	
2	337	29 (8.6)	1.26 (0.68-2.36)	0.461	1.93 (0.96-3.89)	0.066	171	17 (9.9)	2.70 (0.91-8.02)	0.074	2.86 (0.96-8.50)	0.059
3	238	38 (16.0)	2.50 (1.38-4.54)	0.003	5.18 (2.59-10.36)	<0.001	110	21 (19.1)	5.45 (1.87-15.87)	0.002	5.50 (1.89-16.03)	0.002
4	63	13 (20.6)	3.27 (1.55-6.87)	0.002	6.84 (2.96-15.79)	<0.001	24	6 (25.0)	7.51 (2.12-26.62)	0.002	7.53 (2.11-26.84)	0.002
Trend				<0.001		<0.001				<0.001		<0.001
0-2	557	44 (7.9)	1.00		1.00		275	21 (7.6)	1.00		1.00	
3-4	301	51 (16.9)	2.29 (1.53-3.43)	<0.001	3.55 (2.30-5.50)	<0.001	134	27 (20.2)	2.84 (1.61-5.03)	<0.001	2.77 (1.56-4.90)	<0.001

SNP, single nucleotide polymorphisms; CMSS, cutaneous melanoma-specific survival; GWAS, genome-wide association study; MDACC, The University of Texas MD

Anderson cancer center; HR, hazards ratio; CI, confidence interval;

<sup>a</sup>Adjusted for age, sex, Breslow thickness, distant/regional metastasis, ulceration and mitotic rate in Cox models of SNPs and CMSS in MDACC study;

<sup>b</sup>Adjusted for age and sex in Harvard study;

<sup>c</sup>Risk genotypes include *MMP16* rs10090371 CA+AA, *ADAMTS3* rs788935 TC+CC, *TLL2* rs10882807 TC+CC, and *MMP9* rs3918251 AA.

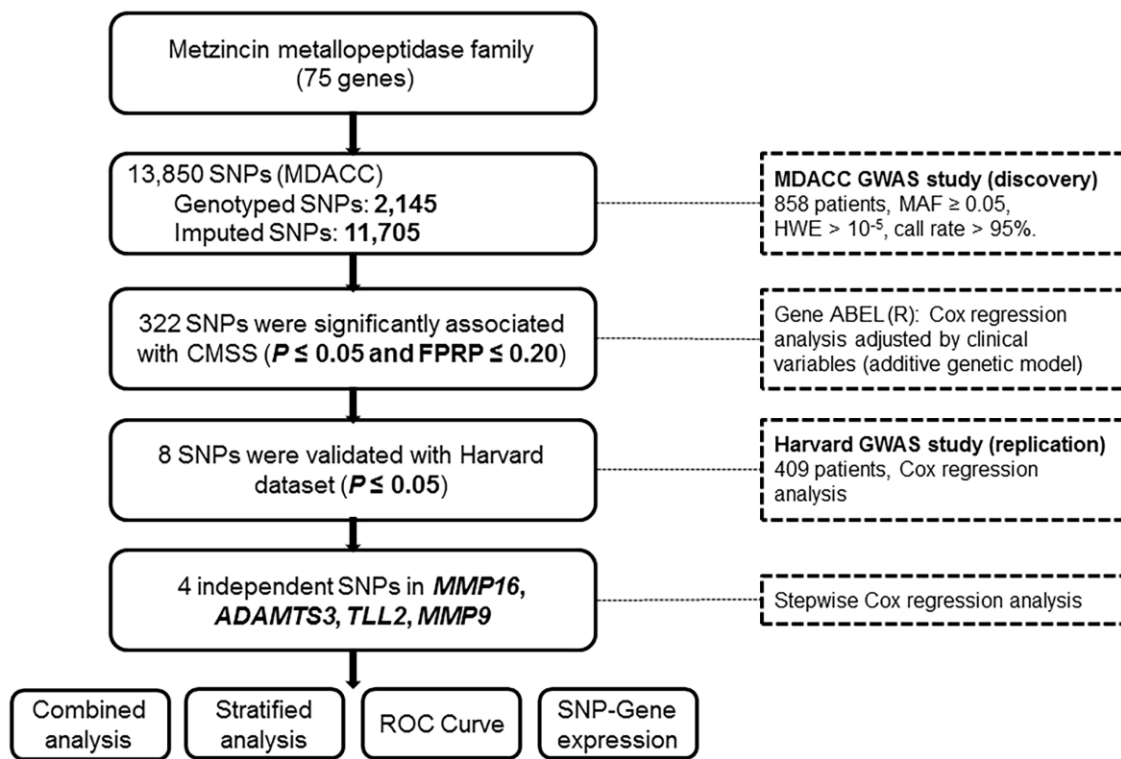


Figure 1

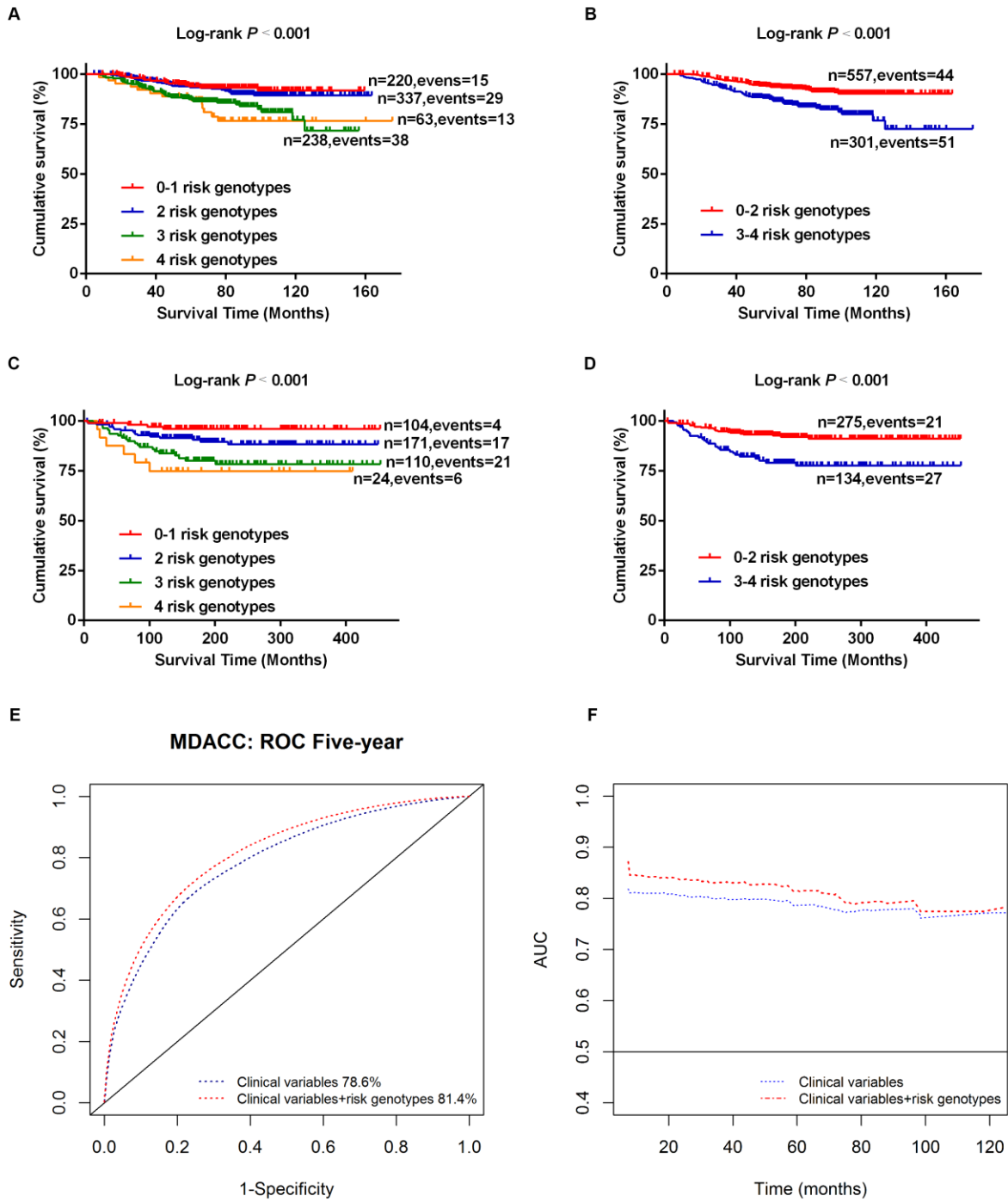


Figure 2