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Genetic variants in the metzincin metallopeptidase family genes predict melanoma survival

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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

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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 ($P_{trend} < 0.001$). 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.

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 endopepdidases 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 metallopeptidase family in melanoma survival. We hypothesize that genetic variants of the metzincin metallopeptidase 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 metallopeptidase 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 metallopeptidase family genes were selected to be analyzed with the following quality control criteria: (1) a genotyping rate ≥ 95%, (2) a minor allelic frequency (MAF) ≥ 0.05, and (3) Hardy-Weinberg equilibrium (HWE) P value ≥ 1×10^{-5}.

Statistical analysis

CMSS was considered the major end-point in 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 metallopeptidase 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 ≤ 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 CMSS associated with the genetic score ($P_{trend} < 0.001$ 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 favorite 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 metallopeptidase with thrombospondin type 1 motif 3. As one of adamlysins 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 metallopeptidase 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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 metallopeptidase 9
ADAMTS	a disintegrin-metalloproteinases with thrombospondin domains
ADAMTS3	ADAM metallopeptidase with thrombospondin type 1 motif 3
TLL2	tolloid-like protein 2

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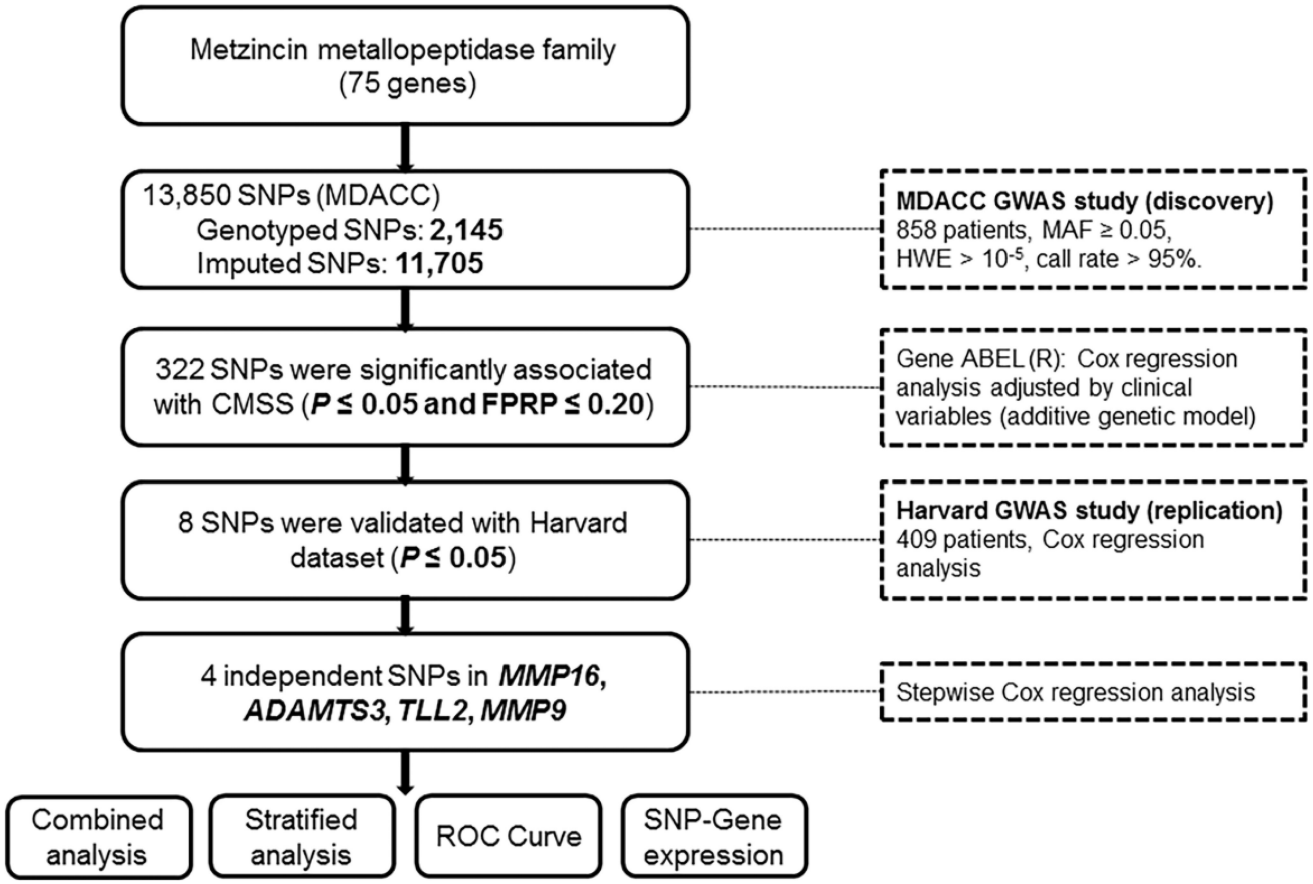

Figure 1.

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.

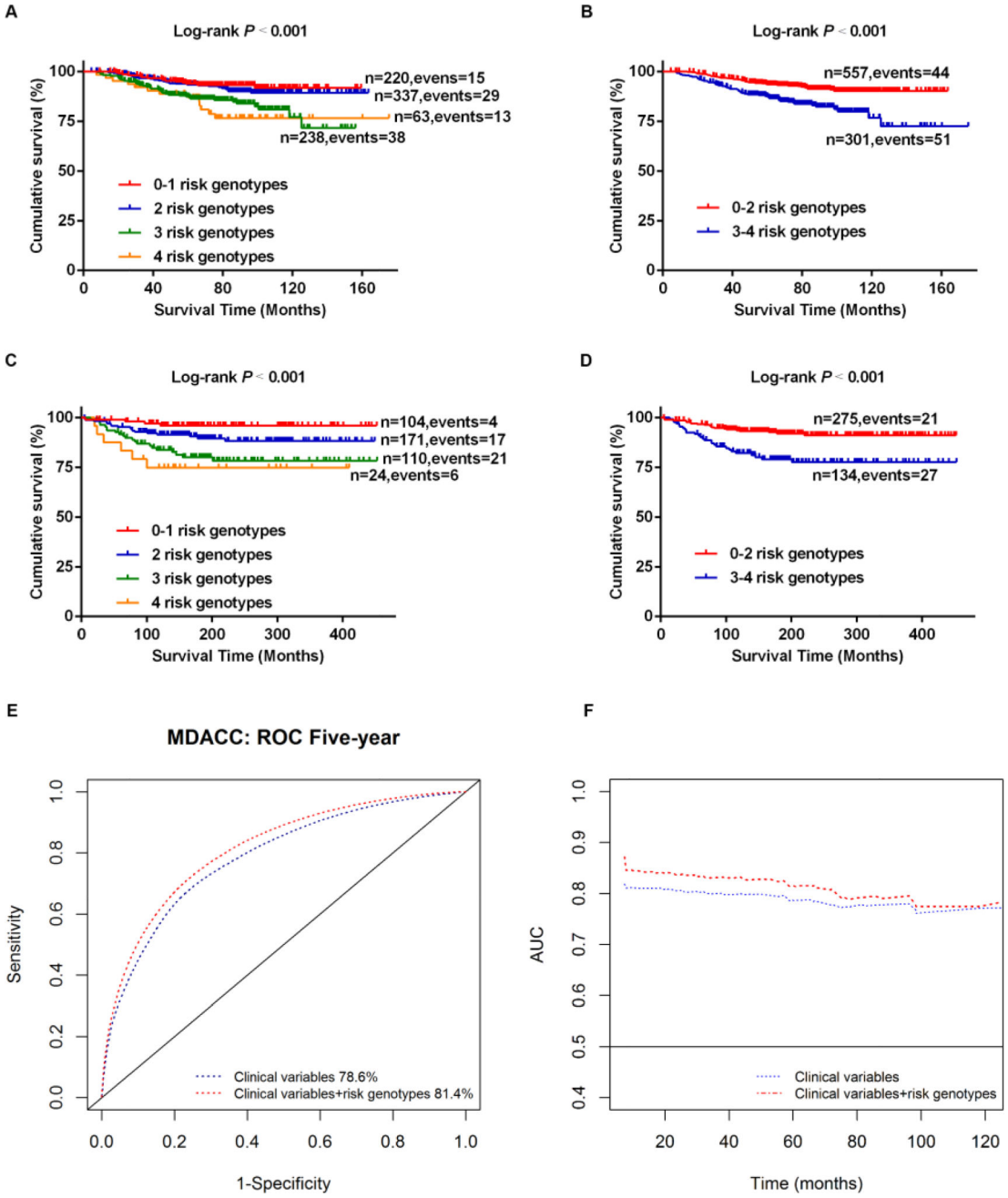

Figure 2.

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.

Table 1

Meta-analysis of eight validated SNPs using two published melanoma GWAS datasets

SNP	Allele[a]	Gene	MDACC (n=858)			Harvard (n=409)			Meta-analysis				Prior probability[f]				
			EAF	HR (95%CI)[b]	*P*[b]	EAF	HR (95%CI)[c]	*P*[c]	P_{het}[d]	I^2	HR (95%CI)[e]	*P*[e]	0.25	0.1	0.01	0.001	0.0001
rs10090371	C/A	*MMP16*	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	*MMP16*	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	*MMP16*	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	*MMP16*	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	*ADAMTS3*	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	*ADAMTS3*	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	*TLL2*	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	*MMP9*	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;

[a] Reference allele/effect allele;

[b] 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 ($P > 0.05$);

[c] Adjusted for age and sex in Harvard study;

[d] P_{het}: *P* value for heterogeneity by Cochrane'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[a]	Category[b]	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^2)	≤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;

[a] 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*);

[b] 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[a]		Frequency		Univariate analysis		Multivariate analysis[b]	
	All	Death (%)	HR (95%CI)	*P*	HR (95%CI)	*P*	All	Death (%)	HR (95%CI)	*P*	HR (95%CI)	*P*
***MMP16* rs10090371 C>A**												
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
***ADAMTS3* rs788935 T>C**												
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
***TLL2* rs10882807 T>C**												
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
***MMP9* rs3918251 A>G**												
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

Genotype	MDACC						Harvard					
	Frequency		Univariate analysis		Multivariate analysis[a]		Frequency		Univariate analysis		Multivariate analysis[b]	
	All	Death (%)	HR (95%CI)	*P*	HR (95%CI)	*P*	All	Death (%)	HR (95%CI)	*P*	HR (95%CI)	*P*
Number of risk genotypes[c]												
0	33	2 (6.1)	1.00		1.00		10	0 (0.0)	1.00		1.00	
1	187	13 (7.0)					94	4 (4.3)				
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;

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

[b]Adjusted for age and sex in Harvard study;

[c]Risk genotypes include *MMP16* rs10090371 CA+AA, *ADAMTS3* rs788935 TC+CC, *TLL2* rs10882807 TC+CC, and *MMP9* rs3918251 AA.