HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2015 August 01.

Published in final edited form as:

J Invest Dermatol. 2015 February ; 135(2): 542–550. doi:10.1038/jid.2014.416.

Genetic variants in Fanconi Anemia Pathway Genes *BRCA2* and *FANCA* Predict Melanoma Survival

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Abstract

Cutaneous melanoma (CM) is the most lethal skin cancer. The Fanconi Anemia (FA) pathway involved in DNA crosslinks repair may affect CM susceptibility and prognosis. Using data derived from published genome-wide association study, we comprehensively analyzed the associations of 2339 common single nucleotide polymorphisms (SNPs) in 14 autosomal FA genes with overall survival (OS) in 858 CM patients. By performing false-positive report probability corrections and stepwise Cox proportional hazards regression analyses, we identified significant associations between CM OS and four putatively functional SNPs: *BRCA2* rs10492396 [AG vs. GG: adjusted hazard ratio (adjHR)=1.85, 95% confident interval (CI)=1.16-2.95, *P*=0.010], rs206118 (CC vs. TT+TC: adjHR=2.44, 95% CI=1.27-4.67, *P*=0.007), rs3752447 (CC vs. TT+TC: adjHR=2.10, 95% CI=1.38-3.18, *P*=0.0005), and *FANCA* rs62068372 (TT vs. CC+CT: adjHR=1.85, 95%

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CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

CI=1.27-2.69, *P*=0.001). Moreover, patients with an increasing number of unfavorable genotypes (NUG) of these loci had markedly reduced OS and melanoma-specific survival (MSS). The final model incorporating with NUG, tumor stage and Breslow thickness showed an improved discriminatory ability to classify both 5-year OS and 5-year MSS. Additional investigations, preferably prospective studies, are needed to validate our findings.

Keywords

cutaneous melanoma; Fanconi Anemia pathway; survival; single nucleotide polymorphisms; Cox regression

INTRODUCTION

In contrast to the stable or declining trends for most cancer types, the incidence of cutaneous melanoma (CM) is increasing in the United States (Siegel *et al.*, 2014), where approximately 76100 CM and additional 63770 *in situ* cases are expected to occur with 9710 deaths in 2014 (Siegel *et al.*, 2014). This increase can be partly ascribed to increasingly sensitive and effective screening, as reflected by the decreasing mean tumor thickness (Lens and Dawes, 2004); nevertheless, there has been little improvement in accurately assessing patient prognosis, because CM still has a heterogeneous prognosis, and the overall 5-year CM survival rate varies substantially among patients, from 15% for distant metastasis to about 98% for localized CM (Balch *et al.*, 2009).

Current prognostic tools mainly included clinicopathological variables, such as tumor stage and Breslow thickness (Balch *et al.*, 2009). However, these methods have insufficient discriminative ability for personalized clinical assessment (Schramm and Mann, 2011). For example, sentinel lymph node biopsy (SLNB) has emerged as an effective and powerful strategy for staging the regional lymphatics in intermediate-thickness CM, yet its prognostic role in thin CM remains somewhat controversial (Kupferman *et al.*, 2014; Sabel, 2012). Meanwhile, it remains difficult to establish prognostic models for CM patients with age <20 years (Sanlorenzo *et al.*, 2013). These call for development of additional or better markers with specific prognostic potential, allowing for personalized healthcare. There is growing evidence for a role of genetic (germline) variants in CM prognosis (Li *et al.*, 2013; Liu *et al.*, 2012; Rendleman *et al.*, 2013), which may lead to improved prediction of prognosis. Discovery of such genetic variants might also provide clues about the mechanisms underlying melanocyte carcinogenesis and CM progression.

Fanconi Anemia (FA) is an inherited disease associated with bone marrow failure, progressive pancytopenia and multiple developmental defects and characterized by chromosomal instability, cancer susceptibility and exquisitely sensitivity to agents that produce DNA interstrand cross-links. The FA pathway consists of at least 14 complementation groups [i.e., FANC-A, B, C, D1 (BRCA2), D2, E, F, G, I, J, L, M, N and P (BTBD12)] and one FA-like complementation group (FANCO) (Crossan and Patel, 2012). In brief, the eight upstream FA proteins assemble into a core complex (FANC-A, B, C, E, F, G, L, and M), and then mono-ubiquitylate its two substrates, FANCD2 and FANCI. Consequently, the ubiquitinated FANCD2/FANCI complex is directed to the nucleus where

it binds to chromatin and recruits the downstream FA proteins (FANC-D1, N, J) and additional DNA repair proteins (i.e., BRCA1) (Crossan and Patel, 2012; Stoepker *et al.*, 2011). These downstream members participate in DNA repair by homologous recombination (Moldovan and D'Andrea, 2009). The main role of FA proteins is to repair DNA cross-links (Kennedy and D'Andrea, 2005). Additionally, the FA pathway can promote stem-cell function, stabilize replication forks, prevent tumorigenesis and inhibit inaccurate repair (Kennedy and D'Andrea, 2005; Kottemann and Smogorzewska, 2013).

Although ultraviolet light induces DNA lesions, disrupts genetic integrity and contributes to CM susceptibility (von Thaler *et al.*, 2010), the host DNA repair capacity may also affect treatment efficacy and resistance to certain chemotherapeutic regimens and thereby affect malignant progression and patient survival (Chin *et al.*, 2006; Emmert and Kraemer, 2013; Munshi *et al.*, 2005). For example, genetic variants of nucleotide excision repair genes affect CM survival (Li *et al.*, 2013). Here, we also hypothesize that genetic variations in the FA pathway genes may also modulate clinical outcome of CM patients. In the present study, we tested our hypothesis by using genotyping data of single nucleotide polymorphisms (SNPs) in the FA pathway genes from a previously published genome-wide association study (GWAS) of CM (Amos *et al.*, 2011). We evaluated associations of prognosis in non-Hispanic CM patients with common SNPs in the 14 autosomal genes, with one exception for *FANCB* that is located in X chromosome.

RESULTS

Patient characteristics

As previously described, this study included 858 patients with primary CM (Table S1), who had complete information about clinical variables, questionnaire data and GWAS data (Li et al., 2013). The patients were aged between 17 and 94 years at diagnosis (52.4 ± 14.4 years). There were more stages I/II patients (709, 82.6%) than stages III/IV patients (149, 17.4%). The patients were with a median follow-up time of 81.1 months, during which 133 (15.5%) had died for all reasons at the last follow-up. Among these deaths, 95 died of CM. In the multivariate analyses, six variables were found to be independently and significantly associated with OS, including age at diagnosis, Clark level, tumor stage, Breslow thickness, SLNB and mitotic rate.

Multivariate analysis of SNPs and CM OS

To test the associations of 321 genotyped and 2018 imputed SNPs with OS (Table S2), we performed multivariate Cox proportional hazards regression. As shown in Figure S1, 138 SNPs were individually significantly associated with OS at $P<0.05$ in an additive model, of which 77 SNPs were still considered noteworthy after the correction by the false positive report probability (FPRP) and 15 of these 77 SNPs were predicted to be functional, based on the *in silico* functional prediction by using SNPinfo. These 15 SNPs included seven SNPs of *BRCA2*, seven SNPs of *FANCA*, and one SNP of *BTBD12* (Table S3).

FA pathway variants as independent survival risk factors

Initial stepwise Cox proportional hazards regression analyses suggested four SNPs (*BRCA2* rs10492396 G>A, rs206118 T>C, and rs3752447 C>T and *FANCA* rs62068372 T>C) as independent predictors for OS of CM patients (Table 1 and Table S3). In multivariate Cox proportional hazards regression analyses using an additive model, HR for rs206118 C was 1.40, while rs3752447 T and rs62068372 C showed protective affect against death (Table 2). In recessive models, rs10492396 (only one subject with AA) genotype showed a strong association with shorter OS [AG vs. GG: adjusted hazards ratio (adjHR)=1.85, 95% confident interval (CI)=1.16-2.95, *P*=0.010]. Patients with rs206118 CC exhibited significantly increased hazards of early death, compared with those who had TT+TC genotypes (adjHR=2.44, 95% CI=1.27-4.67, *P*=0.007). Additionally, the rs3752447 CC genotype had a statistically significant impact on OS, compared with TT+TC genotypes (adjHR=2.10, 95% CI=1.38-3.18, *P*=0.0005). Furthermore, rs62068372 was also associated with unfavorable OS, with an HR of 1.85 (TT vs. CC+CT: 95% CI=1.27-2.69, *P*=0.001). For melanoma-specific survival (MSS), rs3752447 CC or rs62068372 TT were more likely to be associated with MSS, compared with other genotypes (adjHR=2.02 and 1.79, respectively); and rs206118 CC was marginally associated with MSS (adjHR=2.12, *P*=0.057, compared with CT+TT), but no significant association was observed between rs10492396 and MSS (Table 2). Table S4 showed the correlation coefficients between these four SNPs, indicating that the effects of SNPs are mostly independent.

Survival of melanoma patients with unfavorable genotypes

When we combined the risk genotypes of rs206118, rs10492396, rs3752447 and rs62068372 in *FANCA* and *BRCA2* into one variable as the number of unfavorable genotypes (NUG), the frequencies of 0, 1, 2, 3/4 NUG were 116, 357, 322 and 62 (there was only 1 patient carrying 4 NUG), respectively. As illustrated in Table 3, per-unit increase of NUG was associated with a reduced OS (adjHR=1.91, 95% CI=1.53 2.39, P_{trend}<0.0001) and MSS (adjHR=1.79, 95% CI=1.38-2.31, P_{trend}<0.0001), respectively. Prognosis was worst in patients with 3/4 NUG for OS (adjHR=7.49; 95% CI=3.36-16.70, *P*<0.0001) and for MSS (adjHR=4.65; 95% CI=1.81-11.91, *P*=0.001).

We next dichotomized all patients into a low-risk group (with 0-1 NUG) and a high-risk group (with 2-4 NUG). We found that compared with the low-risk group, the high-risk group died at twice due to all causes (adjHR=2.41, 95% CI=1.67-3.48, *P*<0.0001) or CM (adjHR=2.53, 95% CI=1.65-3.89, *P*<0.0001). For illustrative purpose, Kaplan Meier curves of the associations with OS, MSS and NUG are shown in Figure 1.

Stratified analyses for NUG with CM Survival

For stage-specific and thickness-specific associations between NUG and CM survival (Table 4), we found that the high-risk genotype group, but not the low-risk genotype group, showed remarkably increased risk of death in those who had Breslow thickness >1 mm, particularly for those with Breslow thickness >4 mm. Stratified analyses revealed that there were no significant differences among strata of tumor stage and thickness status (*P* for heterogeneity >0.100).

Receiver operating characteristic curve

Using multivariate logistic regression and receiver operating characteristic curve, we further evaluated the NUG for its potential to improve the classification of 5-year OS (N = 749; 133 died and 615 alive, and 5-year MSS (N = 732; 95 died due to CM). As shown in Figure 2, including only tumor stage and Breslow thickness as classifiers, the 5-year OS model had an area under the curve (AUC) =73.6%; with the addition of NUG, the AUC was significantly improved to 76.8% (P=0.001, DeLong's test). With tumor stage and Breslow thickness as classifier, the 5-year MSS had an AUC of 80.6%, which improved to 82.8% after adding NUG (P=0.025, DeLong's test). This suggests a potential role of the NUG in prediction of patients at risk for death.

Bioinformatics analyses

By using the existing expression data in melanoma patients from two studies from the public Gene Expression Omnibus (GEO) database that met the inclusion criterion, we then examined the mRNA levels of *BRCA2* and *FANCA* in GSE3189 (25 normal skin/nevi and 45 primary melanoma tissues) and GSE8401 (31 primary melanoma and 52 melanoma metastasis tissues). Figure 3 shows that *BRCA2* had increased gene expression levels in primary melanoma (P=0.014, GSE3189) and the metastasis (P<0.001, GSE8401). Similar results were found for *FANCA* (both P<0.001).

We further evaluated the correlations between SNPs and their corresponding mRNA expression levels in normal cells, using the published expression data of the HapMap normal lymphoblastoid cell lines. Such expression data were available for *BRCA2* rs206118, rs10492396, and rs3752447. Consistent with the observed associations, the rs3752447 CC genotype was associated with significant higher levels of mRNA expression of *BRCA2*, compared with the TT+TC genotypes (P=0.040); whereas for rs10492396, the AG genotype carriers had a marginally higher *BRCA2* expression than those with the GG genotype (no AA carrier; P=0.073). No significant correlation was found between rs206118 genotypes and *BRCA2* mRNA expression levels (P=0.414) in a recessive model. However, the *BRCA2* mRNA expression levels increased in a linear manner with the increasing number of risk genotypes, when combining rs206118, rs10492396, and rs3752447 (P *trend* = 0.019, Figure 4). We did not find any significant result in an additive model (Figure S2), which supports our findings in the risk associations that follow a recessive genetic model.

DISCUSSION

In the present study, we found that *BRCA2* rs206118 T>C, rs10492396 G>A, and rs3752447 C>T and *FANCA* rs62068372 T>C were likely to independently or jointly modulate survival of CM patients and that the incorporation of numbers of risk genotypes of *FANCA* and *BRCA2* could significantly improve the prediction of CM OS and MSS. These findings are biologically plausible, because FA proteins function at different steps in the sensing, recognition and processing of DNA cross links (Kottemann and Smogorzewska, 2013).

An activated FA pathway can provide resistance to increased endogenous DNA damage and confer survival advantage to melanoma cells (Nitta *et al.*, 2010). Evidence also exists that

the FA pathway may also influence cancer treatment and prognosis. For example, a 44-gene microarray-based assay for the FA/BRCA pathway could discriminate two different prognostic groups in breast cancer patients who were treated with adjuvant 5-fluorouracil, epirubicin, and cyclophosphamide (Mulligan *et al.*, 2014). The absence of the mouse *Fancd2* gene product could confer radiosensitivity to bone marrow stromal (Berhane *et al.*, 2014). While high *FANCD2* mRNA expression was a significant independent factor for lymph node metastasis in colorectal cancer (Ozawa *et al.*, 2010), high FANCD2 protein expression appeared to be prognostically unfavorable for OS of sporadic breast cancer (van der Groep *et al.*, 2008). Furthermore, high nuclear staining for cytoplasmic FANCD2 appeared to be associated with death in sporadic and metastatic human breast cancer patients (Rudland *et al.*, 2010); immunocytochemically stain of FANCD2 was associated with pathologic response to neoadjuvant chemoradiation and OS in patients with esophageal cancer (Alexander *et al.*, 2012). Other FA genes have also been shown to be associated with cancer outcomes. For example, deletion/methylation of *FANCC* was significantly associated with locoregional recurrence/death in patients with head and neck squamous cell carcinoma in one study (Ghosh *et al.*, 2013) and also associated with poor survival in breast carcinoma in another study (Sinha *et al.*, 2008). Ovarian cancer cases with promoter methylation of *FANCF* showed an increased risk of progression-free death, compared with those without methylation (Lim *et al.*, 2008). High immunohistochemical expression of FANCJ was significantly associated with 5-fluorouracil resistance and poor recurrence-free survival in colorectal cancer (Nakanishi *et al.*, 2012). Finally, when restricted to lung cancer patients receiving chemotherapy, the *FANCE* A250T variant could predict patients' OS (Matakidou *et al.*, 2007).

In the present study, we found some striking significant associations of CM OS with genetic variants in *FANCA* and *BRCA2*, although not all of these four SNPs showed a significant association with CM MSS. However, the NUG of these SNPs better predicted CM OS and MSS and discriminated among prognostic groups. Notably, the effect was consistent across different analyses and multiple subgroup comparisons, supporting a robust effect of the NUG on CM survival, regardless of other pathological characteristics.

In the downloaded GEO dataset, we found that expression of both *FANCA* and *BRCA2* were up-regulated in tumor tissues of primary melanomas and melanoma metastases, suggesting a possible contribution of *FANCA* and *BRCA2* to CM progression. The *BRCA2 (FANCD1)* gene is located on 13q12.3, while *FANCA* is mapped to 16q24.3. *BRCA2* and *FANCA* are key members of the FA pathway. One study reported that nine FA genes (including *FANCA* and *BRCA2*), but not other pathway genes, were transcriptionally up-regulated in melanoma tissues, compared with normal skin and non-melanoma skin cancer (Kao *et al.*, 2011). Although few studies have linked *FANCA* and *BRCA2* to CM prognosis, *FANCA* and *BRCA2* have been shown to influence treatment and prognosis in other cancers. For instance, recurrent ovarian carcinomas commonly had increased BRCA2 protein expression post chemotherapy exposure, which could mediate resistance to platinum-based therapies (Swisher *et al.*, 2009); whereas the copy number of *FANCA* might be correlated with poor prognosis of head and neck cancer (Bauer *et al.*, 2008). In addition, *FANCA* mRNA was found to be up-regulated in lung carcinoids with a poor prognosis (Swarts *et al.*, 2013).

Although rs206118, rs10492396, and rs3752447 and rs62068372 were predicted to affect corresponding gene expression by SNPinfo (Xu and Taylor, 2009), we were unable to validate on our own specimens. In publically available expression data of the 270 HapMap lymphoblastoid cell lines derived from diverse populations (Holm *et al.*, 2010), we found that the *BRCA2* mRNA expression levels related with the NUG of rs206118, rs10492396, and rs3752447 of *BRCA2*. This genotype-phenotype correlation provides biological evidence that *BRCA2* expression may be mediated jointly by rs206118, rs10492396, and rs3752447, a possible explanation for the observed association with CM survival.

A major strength of this study is the comprehensive analyses of associations between SNPs in the FA pathway and CM survival with a median follow time of 81.1 months. In our analyses, we adjusted for some important variables that could confound the genetic effect on OS. We also performed FPRP to access the possibility of false positive associations. Our findings demonstrated the potential importance of assessing CM prognosis by combining clinicopathological characteristics with genetic information. The observed improvement of discrimination of CM 5-year OS and MSS supports the prognostic impact of associations and potential clinical applications.

However, the current study has some limitations. Firstly, we did not evaluate the potential effects of different therapies on the outcomes of CM patients, or their potential associations with the identified SNPs, because patients received a wide variety of systemic therapies, often sequentially, but had relatively few outcome events for evaluation, making the stratification not meaningful, if not unfeasible. However, the patients in the present study were recruited before the time that vemurafenib was approved by the FDA for the treatment of advanced melanoma in 2011. Hence, the systemic therapies available for the patients in our analysis could only have been expected to be modestly effective in a minority of advanced melanoma patients. We also performed stratified analyses by tumor stage to minimize the effect of different treatment. The results were consistent in stage I/II and stage III/IV patients, suggesting that the presence of diverse treatment did not have significant impact on CM in our analysis, if any. Secondly, the prognosis predicting model was only built in a non-Hispanic white population; the application to other ethnic groups still needs further investigation. Finally, because of the lack of validation in a similar patient population, the interpretation of our findings should be cautious, until validated by others.

MATERIALS AND METHODS

Study populations

Patients were accrued for a hospital-based case-control study of CM at The university of Texas MD Anderson Cancer Center and the characteristics of these patients have also been described elsewhere (Amos *et al.*, 2011). Among the 1804 patients, three patients were excluded due to loss to follow-up after diagnosis. 943 patients were excluded because of missing questionnaire data that were not collected at the clinic when the patients were seen. Therefore, the final analysis included 858 patients who had complete information for clinical prognostic variables (Table S5). Figure S3 shows our sample chose strategy. All individuals provided a written informed consent under an Institutional Review Board-approved protocol.

SNP genotyping

The genotype data in this study can be accessed by using the Database of Genotypes and Phenotypes (dbGaP) (Mailman *et al.*, 2007), with study accession number phs000187.v1.p1. The detailed genotyping information and data quality control can be found in the previously described GWAS (Amos *et al.*, 2011). Genome-wide imputation was performed using the MACH software based on 1000 Genomes project, phase I V2 CEU data (Li *et al.*, 2010).

SNP selection for the FA pathway analysis

Based on the databases of KEGG (http://www.genome.jp/kegg/) and Biocarta (http://www.biocarta.com/), we selected 14 genes that are located on autosomes from the FA pathway: FANCA, FANCC, BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, BRIP1, FANCL, FANCM, PALB2, RAD51C and *BTBD12*. Genotyped or imputed common SNPs (minor allele frequency ≥ 0.05, genotyping rate ≥ 95%, Hardy-Weinberg equilibrium p-value ≥0.00001, and imputation r^2 ≥0.8) within these genes or their ± 20-kb flanking regions were selected for association analysis. As a result, 321 genotyped SNPs and 2018 imputed SNPs in the FA pathway were extracted from our CM GWAS dataset (Table S2).

FPRP

For all the significant results, we assigned a prior probability of 0.1 to detect a HR of 2.0 for an association with genotypes and alleles of each SNP (Wacholder *et al.*, 2004). Only the significant results with an FPRP value <0.2 were considered noteworthy.

Statistical methods

The OS time was calculated from the date of diagnosis to the date of death from any cause or date of the last follow-up. MSS time was determined from the time of diagnosis until death from CM; individuals who died of causes other than CM were considered to be censored. OS was the primary outcome measure to be evaluated in the present study as we had a relatively enough number of the events. Firstly, associations between SNPs and OS (in an additive model) were obtained by multivariable Cox proportional hazards regression analyses performed with GenABEL package of R software (Aulchenko *et al.*, 2007) with adjustment including age, sex, tumor stage, Breslow thickness, SLNB, Clark level, tumor cell mitotic rate and ulceration of tumor (Balch *et al.*, 2009)]. We also applied a FPRP cut-off of 0.2 to limit the probability of false positive findings as a myriad of SNPs had been tested. The significant and functional SNPs were identified by using the FPRP correction and SNPinfo (Xu and Taylor, 2009) and then were included with clinical prognostic variables into a multivariable stepwise Cox proportional hazards regression model. We summarized the number of risk genotypes identified from the stepwise regression models for CM OS. Kaplan-Meier survival curves and log-rank tests were used to evaluate the effects of genetic variants on the cumulative probability of OS. Multiple Cox proportional hazards regression models were also used for stratified analyses by tumor Breslow thickness and stage. The heterogeneity between subgroups was assessed with the Chi-square-based *Q* test and the heterogeneity was considered significant when $P < 0.100$. Receiver operating characteristic curve was constructed from the logistic regression model, and the AUC was used to assess the classification performance of the model. Statistical significance of the

improvement in AUC after adding an explanatory factor was calculated by the Delong's test (DeLong *et al.*, 1988). Second, we also investigated whether significant associations between the variants and OS remained for MSS. Finally, to provide a biological context for our findings, we searched the GEO database (http://www.ncbi.nlm.nih.gov/geo/) for studies that provided mRNA expression data from melanoma patients. The search terms were "melanoma" or "cutaneous melanoma" in combination with "human [organism]", limiting to sample sizes of more than 20. Linear regression analysis was also used to test for the trends in the associations between SNPs and corresponding gene expression levels obtained from the 270 lymphoblastoid cell lines from CEU and other HapMap samples (Holm *et al.*, 2010). All other analyses were performed using SAS software (Version 9.3; SAS institute, Cary, NC).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEGEMENTS

We thank the individuals who participated in this project. We thank the John Hopkins University Center for Inherited Disease Research for conducting high-throughput genotyping for this study. Jieyun Yin is sponsored by the China Scholarship Council for studying at Duke University. This work was supported by the National Institutes of Health, National Cancer Institute Grants R01 grant CA100264, the National Cancer Institute SPORE grant P50 CA093459 and the Marit Peterson Fund for Melanoma Research. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviation

CM	cutaneous melanoma
FA	Fanconi Anemia
SNPs	single nucleotide polymorphisms
OS	overall survival
MSS	melanoma-specific survival
adjHR	adjusted Hazards Ratio
CI	confident interval
SLNB	sentinel lymph node biopsy
FPRP	false positive report probability
AUC	area under the curve
NUG	number of unfavorable genotypes

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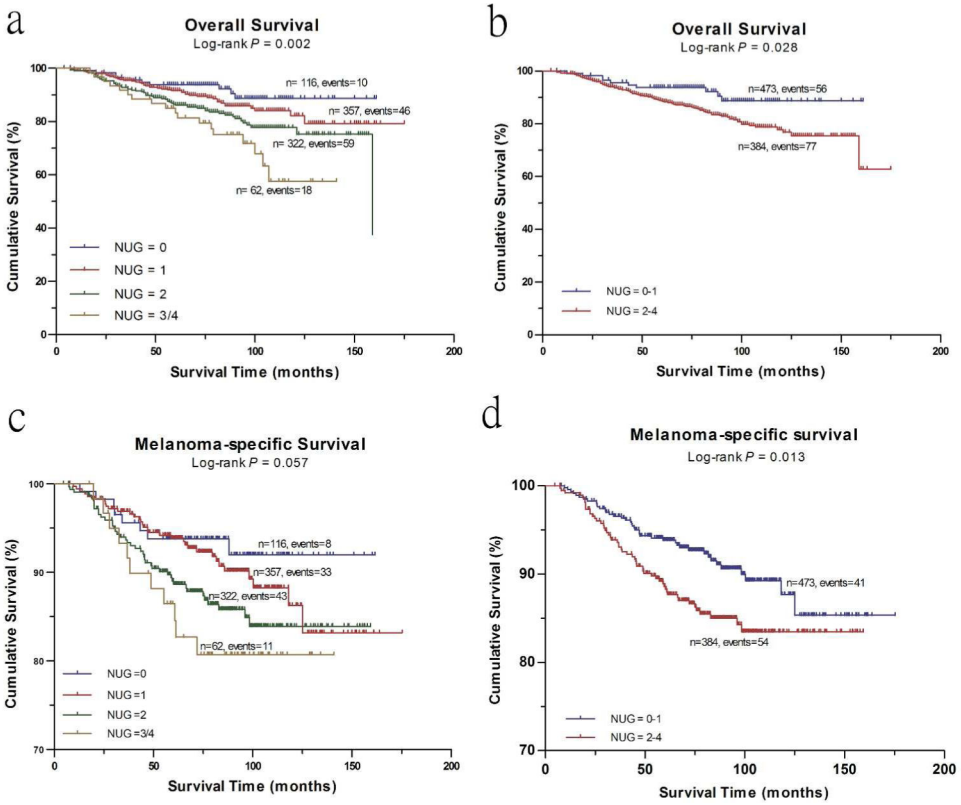

Figure 1. Kaplan-Meier survival analysis

For CM OS (**a**) by 0, 1, 2, 3/4 NUG (i.e., rs10492396 AG, rs206118 CC, rs3752447 CC, rs62068372 TT) and (**b**) by 0-1 and 2-4 NUG; for CM MSS (**c**) by 0, 1, 2, 3/4 NUG and (**d**) by 0-1 and 2-4 NUG.

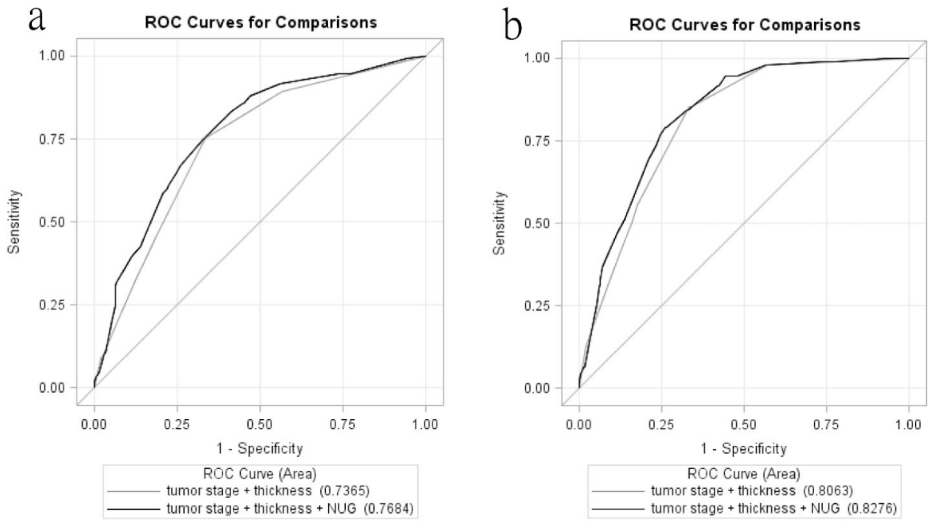

Figure 2. Receiver operating characteristic curves for prediction of CM survival

(**a**) 5-year OS rate and (**b**) 5-year MSS rate, based on tumor stage and thickness; tumor stage, Breslow thickness plus NUG.

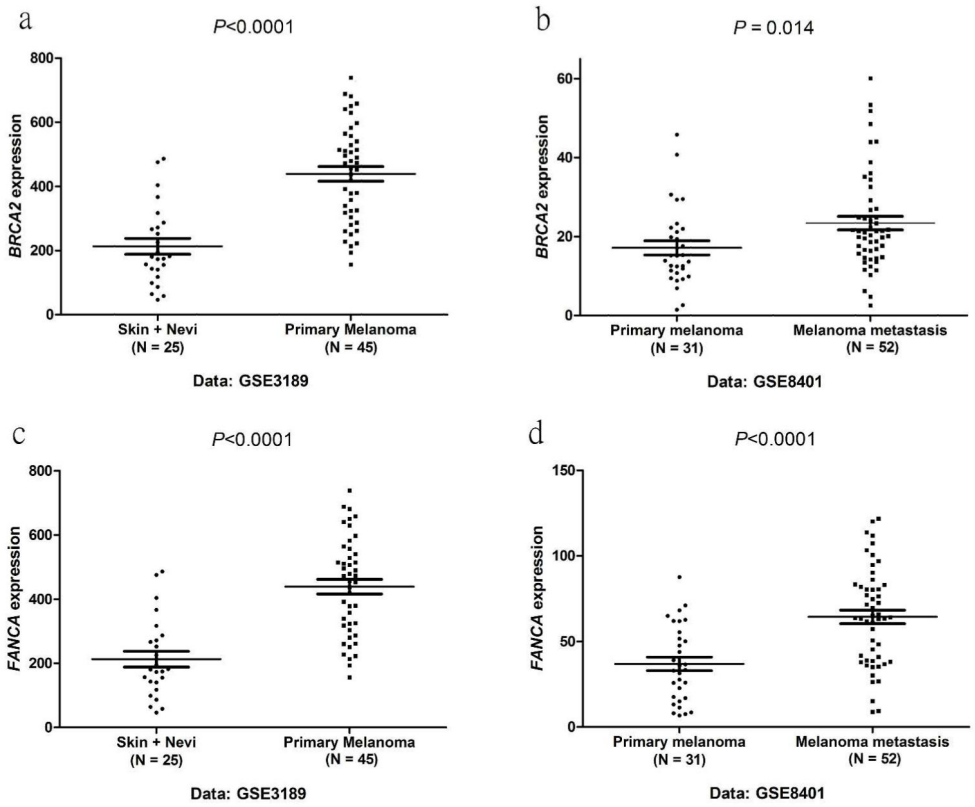

Figure 3. Up-regulation of *BRCA2* (a, b) and *FANCA* (c, d) in the progression of melanoma

The Y axis is a score representing the expression levels of *BRCA2* and *FANCA*.

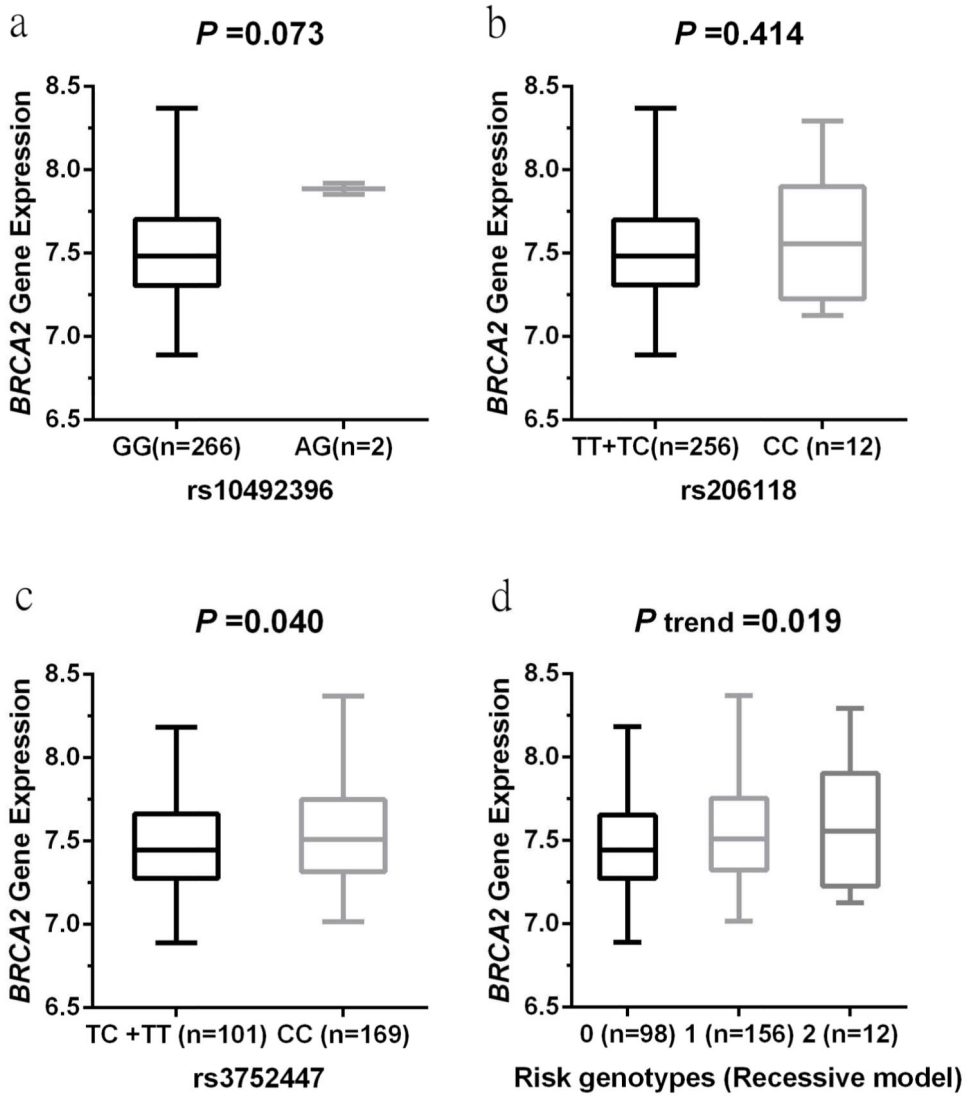

Figure 4. SNP-gene associations

Analyses of BRCA2 expression levels, by genotypes of (**a**) rs10492396, (**b**) rs206118, (**c**) rs3752447 and (**d**) combined risk genotypes (i.e., rs206118 CC, rs10492396 AG, rs3752447 CC) in 270 HapMap lymphoblastoid cell lines from all population. The number of individuals with missing data for rs10492396, rs206118 and combined risk genotypes were 2, 2 and 4, respectively. The Y axis is the normalized gene expression levels.

Table 1

Predictors of OS in CM patients in stepwise Cox regression analysis of selected variables[†]

Parameter	Category[a]	No.	*P* value	HR	95% CI
Age	>50/≤50	487/371	<0.0001	1.05	1.03-1.06
Sex	Female/Male	361/496	0.009	0.59	0.40-0.87
Breslow thickness	>4/1-4/≤1 mm	68/443/347	<0.001	1.10	1.04-1.16
Tumor stage	III+IV/I+II	149/709	<0.0001	3.40	2.30-5.04
Clark level	IV+V/II+III	459/399	0.009	1.85	1.17-2.92
Tumor cell mitotic rate	≥1/<1/mm^2	583/275	0.040	1.75	1.03-2.99
Ulceration of tumor	Yes/No	155/681	<0.0001	2.27	1.56-3.30
rs206118	CC/TT+TC	32/826	0.017	2.24	1.16-4.32
rs10492396	AG/GG	102/755	0.011	1.85	1.16-2.97
rs3752447	CC/TT+TC	589/269	<0.001	2.06	1.35-3.13
rs62068372	TT/CC+CT	467/391	0.002	1.81	1.24-2.63

OS = overall survival; CM = cutaneous melanoma; HR = hazards ratio;

[†]Age, sex, tumor stage, Breslow thickness, SLNB, Clark level, ulceration of tumor, tumor cell mitotic rate, rs206118, rs15869, rs10492397, rs1207952, rs10492396, rs17692629, rs3752447, rs8061528, rs57119673, rs62068372, rs56112321, rs34141697, rs8056353, rs56048434, rs62068387, and rs62068388 genotypes were included in the stepwise multivariate Cox proportional hazards regression analysis;

[a]The "/ category" was used as the reference.

Table 2

Associations between survival of CM patients and selected SNPs in the FA pathway

Genotype	No. of patients	OS					MSS				
		Death (%)	Univariate analysis		Multivariate analysis*		Death (%)	Univariate analysis		Multivariate analysis*	
			HR (95% CI)	*P*	HR (95% CI)	*P*		HR (95% CI)	*P*	HR (95% CI)	*P*
BRCA2 rs10492396 (imputed)											
GG	755	110 (14.6)	1.00		1.00		81 (84.4)	1.00		1.00	
AG	102	23 (22.6)	1.55 (0.99-2.44)	0.055	1.85 (1.16-2.95)	0.010	14 (14.6)	1.31 (0.74-2.31)	0.349	1.52 (0.85-2.71)	0.158
AA	1										
rs206118 (genotyped)											
TT	583	88 (15.1)	1.00		1.00		61 (64.2)	1.00		1.00	
TC	243	35 (14.4)	0.94 (0.64-1.39)	0.764	1.16 (0.77-1.75)	0.484	27 (28.4)	1.04 (0.66-1.64)	0.859	1.25 (0.77-2.02)	0.369
CC	32	10 (31.3)	2.30 (1.20-4.43)	0.013	2.53 (1.31-4.90)	0.006	7 (7.4)	2.29 (1.05-5.01)	0.038	2.26 (1.03-4.97)	0.043
Trend			1.21 (0.90-1.62)	0.208	1.40 (1.04-1.87)	0.027		1.27 (0.9-1.78)	0.177	1.39 (0.99-1.96)	0.058
CC vs. TT+TC			2.34 (1.23-4.47)	0.001	2.44 (1.27-4.67)	0.007		2.26 (1.05-4.88)	0.038	2.12 (0.98-4.62)	0.057
rs3752447 (imputed)											
CC	589	102 (17.3)	1.00		1.00			1.00		1.00	
CT	243	29 (11.9)	0.63 (0.42-0.96)	0.03	0.49 (0.32-0.75)	0.001	72 (75.8)	0.7 (0.43-1.13)	0.141	0.53 (0.32-0.87)	0.011
TT	26	2 (7.7)	0.41 (0.10-1.66)	0.211	0.34 (0.08-1.39)	0.134	22 (23.2)	0.29 (0.04-2.12)	0.224	0.21 (0.03-1.52)	0.122
Trend			0.63 (0.44-0.92)	0.015	0.51 (0.35-0.75)	0.0006	1 (1.1)	0.66 (0.43-1.02)	0.059	0.51 (0.33-0.80)	0.003
CC vs. TT+CT			1.64 (1.09-2.45)	0.017	2.10 (1.38-3.18)	0.0005		1.52 (0.95-2.43)	0.082	2.02 (1.24-3.29)	0.005
FANCA rs62068372 (imputed)											
TT	467	84 (18.0)	1.00		1.00		59 (62.1)	1.00		1.00	
CT	332	38 (11.5)	0.64 (0.44-0.95)	0.025	0.50 (0.33-0.75)	0.001	27 (28.4)	0.66 (0.42-1.04)	0.076	0.51 (0.31-0.82)	0.006
CC	59	11 (18.6)	1.10 (0.59-2.06)	0.765	0.73 (0.38-1.39)	0.336	9 (9.5)	1.26 (0.62-2.54)	0.52	0.81 (0.40-1.67)	0.575
Trend			0.84 (0.63-1.12)	0.229	0.68 (0.50-0.91)	0.011		0.9 (0.64-1.25)	0.515	0.71 (0.50-1.01)	0.058
TT vs. CC+CT			1.41 (0.99-2.00)	0.059	1.85 (1.27-2.69)	0.001		1.33 (0.88-2.02)	0.176	1.79 (1.15-2.77)	0.010

SNP = single nucleotide polymorphisms; FA = Fanconi Anemia; OS = overall survival; CM = cutaneous melanoma; HR = hazards ratio; MSS = melanoma-specific survival;

* Adjusted by age, sex, tumor stage, Breslow thickness, SLNB, Clark level, ulceration of tumor, tumor cell mitotic rate in the Cox models.

Table 3

HRs for associations between survival and NUG across genes in the FA pathway in CM patients

NUG†	No. of patients	OS					MSS				
		Death (%)	Univariate analysis		Multivariate analysis*		Death (%)	Univariate analysis		Multivariate analysis*	
			HR (95% CI)	*P*	HR (95% CI)	*P*		HR (95% CI)	*P*	HR (95% CI)	*P*
0	116	10 (8.6)	1.00		1.00		8 (8.4)	1.00		1.00	
1	357	46 (12.9)	1.55 (0.78-3.07)	0.208	2.02 (1.00-4.08)	0.050	33 (34.7)	1.37 (0.63-2.97)	0.425	1.57 (0.71-3.47)	0.268
2	322	59 (18.3)	2.28 (1.17-4.47)	0.016	3.63 (1.81-7.31)	0.0003	43 (45.3)	2.02 (0.95-4.3)	0.068	3.36 (1.54-7.35)	0.002
3/4[a]	62	18 (29.0)	3.55 (1.64-7.69)	0.001	7.49 (3.36-16.70)	<0.0001	11 (11.6)	2.66 (1.07-6.61)	0.035	4.65 (1.81-11.91)	0.001
Trend test			*P* = 0.0001			*P* <0.0001		*P* = 0.007			*P* <0.0001
0-1	473	56 (11.8)	1.00		1.00		41 (43.2)	1.00		1.00	
2-4	384	77 (20.1)	1.76 (1.25-2.49)	0.001	2.41 (1.67-3.48)	<0.0001	54 (56.8)	1.66 (1.11-2.5)	0.014	2.53 (1.65-3.89)	<0.0001

HR = hazards ratio; NUG = number of unfavorable genotypes; FA = Fanconi Anemia; CM = cutaneous melanoma; OS = overall survival; MSS = melanoma-specific survival;

*Adjusted by age, sex, tumor stage, Breslow thickness, SLNB, Clark level, ulceration of tumor, tumor cell mitotic rate;

†Number of unfavorable genotypes (NUG) included rs206118 CC, rs10492396 AG, rs3752447 CC, and rs62068372 TT;

[a]Only one patient carrying 4 NUG.

Table 4

HRs for associations in stratified analyses between survival and NUG across genes in the FA pathway in CM patients

NUG†	No. of Patients	OS					MSS				
		Death (%)	Log-rank *P*	Multivariate analysis*		P_{het}	Death (%)	Log-rank *P*	Multivariate analysis*		P_{het}
				HR (95% CI)	*P*				HR (95% CI)	*P*	
Tumor stage I/II			0.0002			0.807		0.001			0.388
0-1	376	29 (7.7)		1.00			16 (4.3)		1.00		
2-4	332	56 (16.9)		2.44 (1.53-3.90)	0.0002		35 (10.5)		3.12 (1.66-5.85)	0.0004	
III/IV			0.097					0.135			
0-1	97	27 (27.8)		1.00			25 (25.8)		1.00		
2-4	52	21 (40.4)		2.21 (1.21-4.04)	0.010		19 (36.5)		2.02 (1.08-3.8)	0.029	
Breslow thickness (mm)						0.366					0.938
≤1			0.9 78					0.806			
0-1	187	10 (5.3)		1.00			4 (2.1)		1.00		
2-4	160	9 (5.6)		1.32 (0.48-3.59)	0.589		3 (1.9)		2.36 (0.29-19.19)	0.422	
>1 and ≤ 4			0.0003					0.002			
0-1	243	34 (14.0)		1.00			25 (10.3)		1.00		
2-4	199	57 (28.6)		2.59 (1.65-4.06)	<0.0001		42 (21.1)		2.66 (1.59-4.45)	0.0002	
>4			0.124					0.303			
0-1	43	12 (27.9)		1.00			12 (27.9)		1.00		
2-4	25	11 (44.0)		3.54 (1.28-9.82)	0.015		9 (36.0)		3.04 (1.01-9.14)	0.048	

NUG = number of unfavorable genotypes; FA = Fanconi Anemia; OS = overall survival; MSS = melanoma-specific survival;

*Adjusted by age, sex, tumor stage, Breslow thickness, SLNB, Clark level, ulceration of tumor, tumor cell mitotic rate; P_{het}: *P* values for heterogeneity;

†Number of unfavorable genotypes (NUG) included rs206118 CC, rs10492396 AG, rs3752447 CC, and rs62068372 TT.