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## Genetic Variants in the PIWI-piRNA Pathway Gene *DCP1A* Predict Melanoma Disease-specific Survival

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### Abstract

The Piwi-piRNA pathway is important for germ cell maintenance, genome integrity, DNA methylation and retrotransposon control and thus may be involved in cancer development. In the present study, we comprehensively analyzed prognostic roles of 3,116 common SNPs in PIWI-piRNA pathway genes in melanoma disease-specific survival. A published genome-wide association study (GWAS) by The University of Texas M.D. Anderson Cancer Center was used to identify associated SNPs, which were later validated by another GWAS from the Harvard Nurses' Health Study and Health Professionals Follow-up Study. After multiple testing correction, we found that there were 27 common SNPs in two genes (*PIWIL4* and *DCP1A*) with false discovery rate < 0.2 in the discovery dataset. Three tagSNPs (i.e., rs7933369 and rs508485 in *PIWIL4*;

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#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

rs11551405 in *DCPIA*) were replicated. The rs11551405 A allele, located at the 3' UTR microRNA binding site of *DCPIA*, was associated with an increased risk of melanoma disease-specific death in both discovery dataset [adjusted Hazards ratio (HR) = 1.66, 95% confidence interval (CI) = 1.21–2.27,  $P=1.50\times 10^{-3}$ ] and validation dataset (HR = 1.55, 95% CI = 1.03–2.34,  $P=0.038$ ), compared with the C allele, and their meta-analysis showed an HR of 1.62 (95% CI, 1.26–2.08,  $P=1.55\times 10^{-4}$ ). Using RNA-seq data from the 1000 Genomes Project, we found that *DCPIA* mRNA expression levels increased significantly with the A allele number of rs11551405. Additional large, prospective studies are needed to validate these findings.

### Keywords

cutaneous melanoma; PIWI-piRNA pathway; disease specific survival; single nucleotide polymorphisms; Cox regression

## INTRODUCTION

In recent years, it has become increasingly apparent that non-coding RNAs, especially small regulatory RNAs, play crucial roles in cancer development.<sup>1</sup> To date, three major classes of small regulatory RNAs have been identified, including microRNAs (miRNAs), short-interfering RNAs and PIWI-interacting RNAs (piRNA). piRNA is a novel class of small, endogenous, regulatory, non-coding RNA molecules expressed in animal cells.<sup>2</sup> piRNAs form RNA-protein complexes through binding to PIWI proteins (a subset of Argonaute family proteins). Consequently, these complexes regulate both epigenetic and post-transcriptional gene silencing of transposons and other genetic elements to maintain genome integrity in germline cells<sup>2</sup>, and an abnormal PIWI-piRNA pathway increases repeats of retrotransposons, component parts of telomeres.<sup>3</sup> Initially, most studies showed that piRNAs mainly existed in germline cells,<sup>2</sup> but later, some studies reported a widespread presence of piRNAs in multiple tissues of fruit fly, mouse and rhesus macaque samples,<sup>4</sup> and piRNAs were found to accumulate at the onset of meiosis or during spermatogenesis.<sup>5</sup> Afterwards, piRNAs were also revealed to influence cell proliferation, viability, cell invasion and transwell motility.<sup>1</sup>

In humans, independent studies have accumulated evidence that aberrantly expressed piRNAs may play a role in the biogenesis of different types of cancers, including cancers of the stomach,<sup>6</sup> colon,<sup>7</sup> lung,<sup>7</sup> liver,<sup>8</sup> mesothelium,<sup>7</sup> breast<sup>9</sup> and ovaries<sup>10</sup>. The peripheral blood levels of piRNAs were also suggested to be valuable biomarkers for detecting circulating gastric cancer cells with a favorable area under curve.<sup>11</sup> The functions of PIWI in the germline cells have been extensively investigated; for instance, studies have indicated that altered human PIWI proteins (HIWI and HILI) are aberrantly expressed in a variety of cancers<sup>12–15</sup> and involved in cell growth, adhesion,<sup>15, 16</sup> apoptosis<sup>17</sup> and cancer invasion.<sup>13</sup> There are additional clinical reports suggesting a potential use for PIWI expression in evaluating cancer clinical outcome, such as cancers of the pancreas,<sup>18</sup> colon,<sup>19</sup> esophagus,<sup>20</sup> liver,<sup>21</sup> and stomach<sup>22</sup> as well as gliomas<sup>23</sup> and sarcoma.<sup>24</sup>

Multiple genetic alterations, either germline or somatic, are believed to be involved in cutaneous melanoma (CM) development and progression, and the PIWI-piRNA pathway is

suggested to have been involved in tumorigenesis. Thus, we hypothesize that genetic variants (single-nucleotide polymorphisms, SNPs) of the PIWI-piRNA pathway genes are associated with CM survival. To test this hypothesis, we used available genotyping data of PIWI-piRNA pathway genes from a previously published genome-wide association study (GWAS) of CM,<sup>25</sup> followed by validation in another GWAS dataset from Harvard University.<sup>26</sup> We also tried to provide biological evidence in support for positive associations by performing online gene function prediction<sup>27</sup> and gene expression Quantitative Trait Loci (e-QTL) analyses.

## MATERIALS AND METHODS

### Study populations

**MD Anderson discovery dataset**—As described previously,<sup>25</sup> all patients in the discover GWAS were accrued for a hospital-based case-control study of CM at The University of Texas MD Anderson Cancer Center (MDACC). Characteristic details of the subjects have also been previously described.<sup>28, 29</sup> Briefly, all patients with CM stages I/II (primary tumors without evidence of regional or distant metastasis), stage III (locoregional disease, including in transit, satellite, and/or regional lymph node metastasis), and stage IV (distant metastasis) were classified according to the 7<sup>th</sup> edition of the American Joint Committee on Cancer (AJCC) staging system.<sup>30</sup> Follow-up was conducted according to standardized guidelines.<sup>31</sup> Stage of the disease and length of the follow-up were determined from the date of diagnosis. Among the 1,804 patients, 943 patients were excluded because of no questionnaire data. Three additional patients were excluded due to loss to the follow-up after diagnosis. Hence, the final analysis included 858 patients who had complete information about both questionnaire and clinical prognostic variables. All individuals provided a written informed consent under an Institutional Review Board-approved protocol.

**Harvard validation dataset**—The Harvard dataset consisted of two studies: Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS). Sampling, genotyping and quality control procedures have been described previously.<sup>26</sup> In short, eligible cases in both NHS (317 CM cases) and HPFS (177 CM cases) cohorts were participants with histopathologically confirmed invasive melanoma, diagnosed at any time after baseline up to the 2008 follow-up cycle for both cohorts. These 494 cases with survival data were included in final analysis, and all were US non-Hispanic Caucasians.

### SNP selection and genotyping

Based on the KEGG (<http://www.genome.jp/kegg/>) databases for the piRNAs/PIWI pathway, there were 23 core genes (*TDRKH*, *TDRD5*, *MAEL*, *XRNI*, *DCP1A*, *DDX4*, *TDRD6*, *ASZ1*, *PIWIL2*, *TDRD7*, *TDRD1*, *PIWIL4*, *DDX6*, *PIWIL1*, *RNF17*, *TDRD9*, *TNRC6A*, *TNRC6C*, *PLD6*, *TDRD12*, *PIWIL3*, *TNRC6B* and *MOV10L1*) that are located on autosomes.

Genotyping and quality control (QC) of MDACC genome-wide scan dataset have been previously described.<sup>25</sup> Briefly, genomic DNA extracted from the whole blood was genotyped with the Illumina HumanOmni-Quad\_v1\_0\_B array, and genotypes were called

by using the BeadStudio algorithm, at John Hopkins University Center for Inherited Disease Research (CIDR). Genome-wide imputation was also performed using the MACH software based on the 1000 Genome project, phase I V2 CEPH (Utah residents with ancestry from northern and western Europe) or CEU data. The typed or imputed common SNPs (with a minor allele frequency  $\geq 0.05$ , a genotyping successful rate  $\geq 95\%$ , and a Hardy-Weinberg equilibrium  $P$  value  $\geq 0.001$ , and from imputation for those SNPs with  $r^2 \geq 0.8$ ) within these genes were selected. As a result, 3116 SNPs in 23 PIWI-piRNA pathway genes were extracted from the MDACC GWAS dataset and used for the analyses, of which there were only 105 independent SNPs after performing the LD pruning using SECA with the criterion of  $r^2 < 0.1$ .<sup>32</sup> The Hardy-Weinberg equilibrium  $P$  value for those discussed SNPs in the present study were detailed in Supporting Information Table S1.

Genotyping in the Harvard dataset was performed using the Illumina HumanHap550 array, HumanHap610 array and Affymetrix 6.0 array.<sup>26</sup> Imputation was performed based on genotyped SNPs and haplotype information from phase II HapMap CEU data using the program MACH.<sup>33</sup> Only SNPs with imputation quality  $r^2 > 0.95$  were included, and a total of 1,579,307 SNPs passed through the filter. Finally, we extracted interested SNPs from Harvard dataset for validation.

### Statistical methods

Disease-specific survival (DSS) was the primary endpoint of the present study, which was calculated from the date of diagnosis to the date of death from melanoma or the date of the last follow-up, whichever came first. Using data from the MDACC dataset, associations between SNPs and DSS, presented as hazards ratios (HRs) in an additive model, were obtained by both univariate and multivariate Cox proportional hazards regression models performed with the GenABEL package of R software<sup>34</sup> with adjustment for age, sex, Breslow thickness, tumor stage, tumor cell mitotic rate and ulceration of tumor. A false discovery rate (FDR) cut-off of 0.2 was applied to limit the probability of false positive findings arising from multiple comparisons.<sup>35</sup> Kaplan-Meier survival curves and log-rank tests were also used to evaluate effects of SNPs on DSS. Using linkage disequilibrium (LD) information from the latest 1000 Genomes Project for CEU populations,<sup>36</sup> we selected tagSNPs based on  $r^2 > 0.8$  and LD analysis. Next, the identified tagSNPs were further validated in the Harvard dataset, and pooled HRs and 95% CIs were obtained from the meta-analysis using a conservative random-effects model, and the inter-study heterogeneity was assessed with Cochrane's Q test.

A Fine-Gray<sup>37</sup> competing-risks regression model was further used for univariate and multivariate regression analyses, which results in sub-distribution HR from a proportional hazards model. It assesses the SNPs of interest and cumulative incidence of melanoma-specific death, where deaths due to other causes were modeled as a competing event rather than a censoring event as in a Cox model.

Finally, SNP rs11551405, which was significantly associated with risk of melanoma death in both MDACC and Harvard datasets, was predicted to regulate protein translation by affecting microRNA (miRNA) binding sites activity by SNPinfo.<sup>27</sup> The e-QTL analyses were also used to test for trends in associations between rs11551405 genotypes and

corresponding gene expression levels obtained from RNA-seq data both from all populations and European descendants, which are part of the GEUVADIS RNA sequencing project for the 1000 Genomes Project samples.<sup>38</sup> The GEUVADIS RNA sequencing project for the 1000 Genomes Project samples have combined transcriptome and genome sequencing data generated by mRNA and miRNA sequencing on 465 lymphoblastoid cell lines (LCLs) derived from five populations of the 1000 Genomes Project: the CEPH (CEU), Finns (FIN), British (GBR), Toscani (TSI) and Yoruba (YRI). Of these samples, 423 were part of the 1000 Genomes Project Phase 1 dataset<sup>39</sup> with low-coverage whole genome and high-coverage exome sequencing data, and the remaining 42 are part of the later phases of the 1000 Genomes Project with Omni 2.5M SNP array data available at time of this study; these genotypes were imputed from the array data using Phase 1 as the reference.<sup>40</sup> All other analyses were performed using SAS software (Version 9.3; SAS institute, Cary, NC). All reported *P* values were two-sided, and *P*<0.05 was considered statistically significant. The flow chart of this study is illustrated in Supporting Information Fig S1.

## RESULTS

### Patient characteristics

The final analysis included 858 patients from MDACC and 494 patients from Harvard University (Table 1). All patients with CM were non-Hispanic white, with complete information regarding follow-up and GWAS data. In the MDACC dataset, patients had an age range between 17 and 94 years (with a mean age of  $52.4 \pm 14.4$  years) at diagnosis, with more men (496, 57.8%) than women (362, 42.2%). There were more patients with stages I/II melanoma (709, 82.6%) than with stages III/IV melanoma (149, 17.4%). Pathological information was shown in Supporting Information Table S2. The 858 MDACC patients had a median follow-up time (MFT) of 81 months (95% CI = 82.9–88.7; interquartile range: 67.2–103.0), during which 95 (11.07%) died of CM; while the 494 Harvard patients had a mean age  $60.1 \pm 10.6$  years at diagnosis and a relatively longer MFT (179 months, 95% CI = 177–203; interquartile range: 140.0–287.0), during which 57 (11.5%) patients died of CM.

### Discovery in the MDACC dataset

To assess associations of 3116 SNPs in 23 PIWI-piRNA pathway genes (Supporting Information Table S3) with DSS, we performed both univariate and multivariate Cox hazards regression analyses with adjustment for age, sex, tumor stage, Breslow thickness, ulceration of tumor and tumor cell mitotic rate. Specifically, 257 SNPs were individually significantly associated with DSS at *P*< 0.05 in an additive genetic model (Supporting Information Fig S2). When FDR was used to control the probability of false positive associations arising from multiple comparisons, 27 SNPs were still considered noteworthy, including 19 SNPs of *DCPIA* and 8 SNPs of *PIWIL4* (Supporting Information Fig S2 and Supporting Information Table S4). The regional association plots for *DCPIA* and *PIWIL4* in the additive genetic model are presented in Supporting Information Fig S3. Next, with  $r^2 > 0.8$  among SNPs in the same gene as the cut-off value, *DCPIA* rs11551405 C>A and *PIWIL4* rs7933369 G>A, rs508485 T>C were chosen as the representative tagSNPs (Supporting Information Fig S4). We found that the rs11551405 A allele showed a strong association with a shorter DSS [A vs C: adjusted HR = 1.66, 95% confident interval (CI) =

1.21–2.27,  $P = 1.50 \times 10^{-3}$  in an additive model]. Besides, rs7933369 A allele carriers exhibited a significantly increased HR of early melanoma-specific death (adjusted HR = 1.97, 95% CI = 1.45–2.67,  $P = 1.43 \times 10^{-5}$ ), compared with G allele carriers. Furthermore, the rs508485 C allele was associated with a statistically significantly favorable DSS (adjusted HR = 0.57, 95% CI = 0.42–0.77,  $P = 0.0002$ ), compared with the T allele (Table 2).

### Validation in the Harvard dataset

As shown in Table 2 and Supporting Information Fig S2, the initial Cox regression analyses indicated that three SNPs (*DCPIA* rs11551405 C>A and *PIWIL4* rs7933369 G>A and rs508485 T>C) were important predictors for DSS of CM patients. We further validated the effects on risk of DSS in the Harvard dataset. As shown in Table 3, per-unit increase of the rs11551405 A allele (the trend measure) was associated with an increased risk of melanoma-specific death (HR = 1.55, 95% CI = 1.03–2.34,  $P = 0.038$ ). However, associations with *PIWIL4* rs7933369 A and, rs508485 C failed to reach significance ( $P = 0.767$  and 0.806, respectively).

### Meta-analysis

When we combined the results from the MDACC and the Harvard datasets together, there was no heterogeneity for rs11551405 among the two datasets ( $P_{\text{heterogeneous}} = 0.792$ ), and this SNP was associated with a significantly increased risk of melanoma-specific death by 1.62 fold (95% CI = 1.26–2.08,  $P = 1.55 \times 10^{-4}$ , Supporting Information Fig S5). As shown in Fig. 1 with the Kaplan-Meier curves, patients with an increased number of the *DCPIA* rs11551405 A allele had a poorer DSS (log-rank  $P = 0.003$  in the MDACC dataset, and log-rank  $P = 0.059$  in the Harvard dataset), compared with those with C allele. The results for other two SNPs remained statistically non-significant.

### Competing-risks regression model

In the Fine and Gray competing-risks regression model, cumulative incidence of an event of interest (i.e., melanoma-specific mortality) was calculated in the presence of competing risks (death from other causes). During the follow-up, 38 and 117 patients died as a result of other causes in MDACC and Harvard datasets, respectively. Table 3 lists univariate and multivariate competing-risks regression models. In multivariate competing-risks regression models, rs11551405 was a statistically significant predictor of melanoma-specific death, after accounting for other-cause mortality in both datasets (with a sub-distribution HR of 1.61 in the MDACC and 1.53 in Harvard study populations).

### Expression Quantitative Trait Loci (e-QTL) analyses

We further evaluated correlations between rs11551405 genotypes and mRNA expression levels of *DCPIA* in normal cells, a possible functional basis for the observed associations, by using the gene expression data of the publically available RNA-seq data both from all populations (457 individuals) and European descendants (370 individuals, from the 1000 Genomes Project), whose genotyping data were available for *DCPIA* rs11551405. Consistent with the observed associations, the rs11551405 A allele were associated with

significantly higher mRNA expression levels of *DCPIA* in all populations ( $P = 0.039$ ) and European descendants ( $P = 0.043$ ) in an additive genetic model (Fig. 2).

## DISCUSSION

To our knowledge, this is the first study to evaluate associations between genetic variants in PIWI-piRNA pathway genes and CM DSS. We found that *DCPIA* rs11551405 was likely to modulate DSS of CM patients, possibly through a mechanism of modulating gene expression.

Regulation of mRNA translation and degradation is critical in control of cell growth and survival. Processing bodies (P-bodies) are dynamic foci with untranslated mRNA and can serve as sites of mRNA degradation.<sup>41</sup> The full name of *DCPIA* is mRNA-Decapping Enzyme 1a (Dcp1a), and *DCPIA* is a component of the decapping enzyme and has been shown to reside in P-bodies in mammals.<sup>42</sup> In animal experiments, Dcp1a is also suggested to be involved in modulation of, or signal transduction from, P-bodies.<sup>43</sup> Meanwhile, the turnover of mRNA is a critical step in the regulation of gene expression, and an important step in mRNA decay is removal of the 5' cap.<sup>43</sup> Following the poly(A) tail shortening, the 5-methylguanosine cap is removed through the action of Dcp1a and Dcp2.<sup>42</sup> Subsequent to decapping, Xrn1 degrades mRNA in a 5-3 fashion.<sup>44</sup> Meanwhile, Dcp1a and other proteins involved in mRNA degradation or translation repression are key factors in the messenger ribonucleoprotein granule assembly.<sup>42</sup> Dcp1a has also been reported to have roles in cellular signaling. Dcp1a contains an N-terminal EVH1 (enabled vasodilator-stimulated protein homology 1) domain, and the EVH1 domain of Dcp1a has a role in transforming growth factor- $\beta$  signaling through a SMAD4 interaction.<sup>45</sup> In addition, Dcp1a is found to induce translational arrest through protein kinase R (PKR) activation that requires the EVH1 Domain.<sup>46</sup> Interestingly, in a recent investigation of the status of Dcp1a and P-bodies during stages of the cell cycle, Dcp1a was found to be hyper-phosphorylated during mitosis.<sup>42</sup>

In the present study, we revealed some significant associations between genetic variants in *DCPIA* and CM DSS. Specifically, The *DCPIA* rs11551405 C>A SNP showed a prognostic role in both MDACC and Harvard datasets. This variant was computationally predicted to be located in the binding sites of hsa-miR-1322, hsa-miR-144, hsa-miR-203, hsa-miR-299-3p, hsa-miR-302c, hsa-miR-302d, hsa-miR-338-3p, hsa-miR-372, hsa-miR-373, hsa-miR-520a-3p, hsa-miR-520b, hsa-miR-520c-3p, hsa-miR-520d-3p, hsa-miR-520e, hsa-miR-520f, hsa-miR-522, hsa-miR-573 and hsa-miR-584.<sup>27</sup> Among the above-mentioned miRNAs, miR-203 was reported to be associated with melanoma survival.<sup>47</sup> Therefore, rs11551405 may regulate protein translation by affecting miRNA-binding site activity.<sup>27</sup> Genetic variants in the predicted miRNA binding sites are suggested to be deleterious, and they are likely to be candidates for causal variants of human disease<sup>48</sup> or associated with disease survival.<sup>49</sup> At the same time, in the published expression data of the 1000 Genomes Project,<sup>38</sup> we found that the *DCPIA* mRNA expression levels changed in a linear manner with an increasing number of the rs11551405A allele in an additive genetic model. We also performed FDR multiple comparison correction tests to assess the possibility of false positive associations with adjustment for some statistically significant and clinically important variables that could confound genetic effects on DSS.

In MDACC and Harvard datasets, none of the top five components showed significant association with melanoma-specific survival, so they were not controlled in the survival analyses. Another caveat regarding the present study is that the adjustment in the Harvard dataset only contained age and sex, which were inconsistent with the MDACC dataset, due to limited information available to us. But in the MDACC dataset, we found that the results of baseline model (adjusted for age and sex) and the multivariate model (adjusted for age, sex, tumor stage, Breslow thickness, ulceration of tumor and tumor cell mitotic rate) were similar, suggesting that our results did not dramatically change in different adjustment models. In addition, the identified SNP just has moderate effect on melanoma prognosis, which limited its application to personal prognostic management. Considering this, if could be validated by other independent studies, this SNP needs to be combined with identified genetic factors and clinical variables for personal prognostic assessment.

In summary, we performed a comprehensive assessment of genetic variants in genes involved in the PIWI-piRNA pathway, and we identified that *DCPIA* rs11551405 may have a prognostic effect on survival of CM patients. However, our findings need to be validated in other independent, larger patient populations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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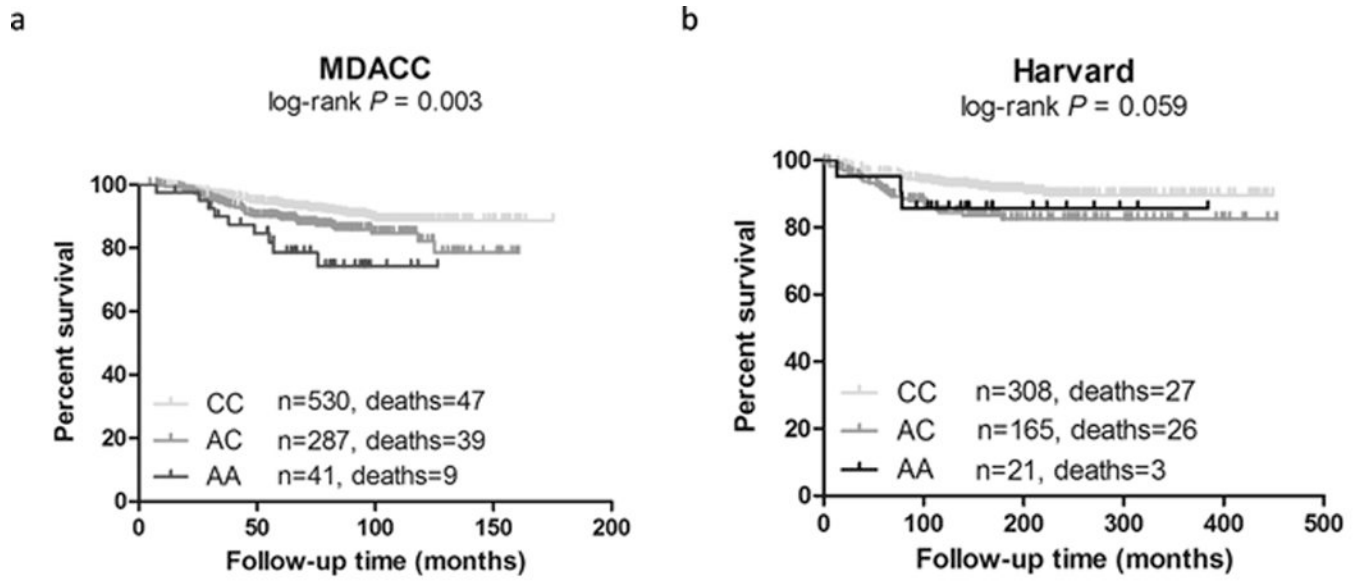
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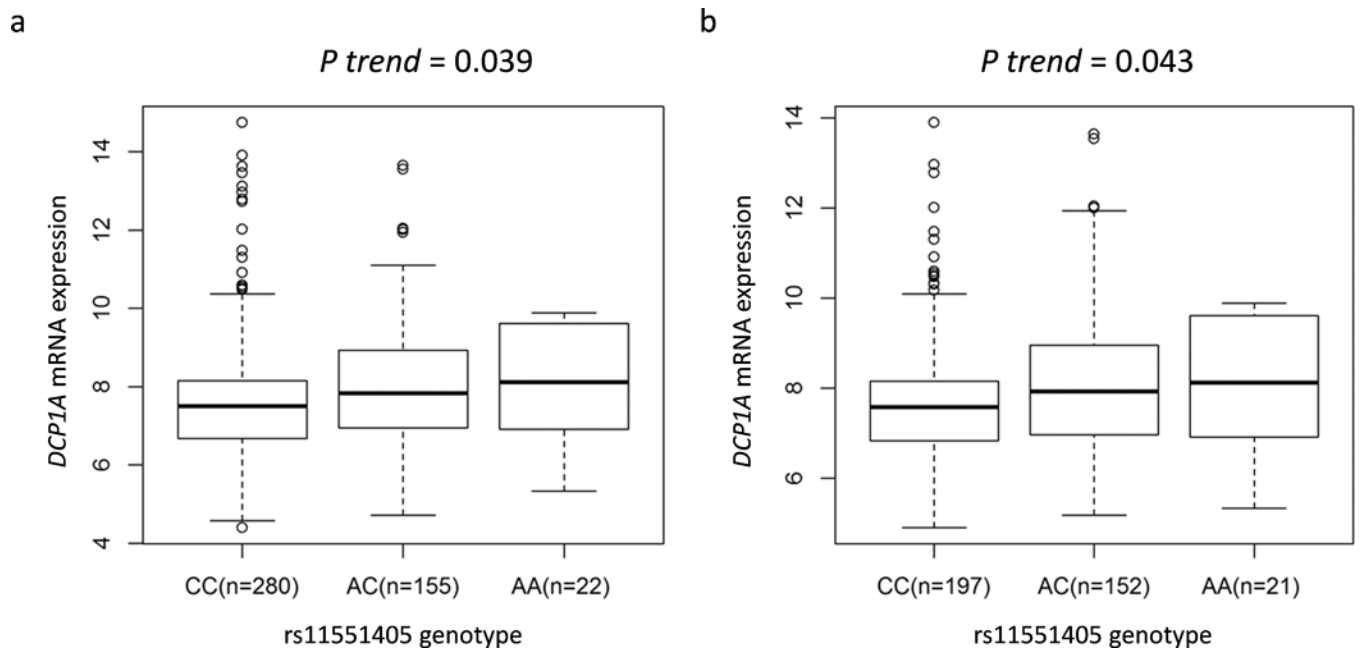
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### Novelty and Impact

The PIWI-piRNA pathway is suggested to be involved in tumorigenesis. In the present study, the authors identified one tagSNP, rs11551405 C>A in the PIWI-piRNA pathway gene *DCPIA*, was associated with an increased risk of melanoma disease-specific death in both discovery and validation datasets. The discovery offers a translational potential for using genetic variants in the PIWI-piRNA pathway gene *DCPIA* as biomarkers for developing improved prognostic assessment and personalized management of cutaneous melanoma patients.



**Figure 1.** Kaplan-Meier survival curve plots of CM DSS with different rs11551405 genotypes in (a) the MDACC dataset and (b) the Harvard dataset.



**Figure 2.**

Association between expression quantitative trait loci (eQTL) and *DCP1A* rs11551405 genotypes. The eQTL analyses were performed in additive models. We used RNA-seq data both from (a) all populations (457 individuals) and (b) European descendants (370 individuals), which are part of the 1000 Genomes Project.

**Table 1**

Characteristics of the study populations at the time of analysis

Parameter	MDACC					Harvard				
	Patients	Death (%)	MFT	HR (95%CI)*	P*	Patients	Death (%)	MFT	HR (95%CI)*	P*
Total	858	95 (11.1)	81.0			494	57 (11.5)	179		
Age (years old)										
50	371	31 (8.4)	85.8	1.00		77	3 (3.9)	340	1.00	
>50	487	64 (13.1)	78.1	1.69 (1.10 – 2.59)	0.017	417	54 (13.0)	161	4.19 (1.31 – 13.46)	0.016
Sex										
Male	496	69 (13.9)	77.8	1.00		177	22 (12.4)	191	1.00	
Female	362	26 (7.2)	85.9	0.48 (0.31 – 0.76)	0.002	317	35 (11.0)	152	1.20 (0.70 – 2.04)	0.508

MDACC, MD Anderson Cancer Center; MFT, median follow-up time (months); HR, hazards ratio; 95%CI, 95% confidence interval;

\* univariate analysis.

**Table 2**

Associations between DSS of CM patients and selected SNPs in the PIWI-piRNA pathway in the MDACC dataset.

Genotype	No. of patients	Death (%)	Univariate analysis		Multivariate analysis*	
			HR (95% CI)	P	HR (95% CI)	P
<i>PIWIL4</i>						
rs7933369						
GG	250	22 (8.8)	1.00		1.00	
AG	427	40 (9.4)	1.07 (0.64–1.80)	0.797	1.44 (0.83–2.51)	0.193
AA	181	33 (18.2)	2.20 (1.28–3.78)	0.004	3.66 (2.04–6.56)	1.31×10 <sup>-5</sup>
Trend			1.53 (1.15–2.05)	0.004	1.97 (1.45–2.67)	1.43×10 <sup>-5</sup>
<i>PIWIL4</i>						
rs508485						
TT	224	34 (15.2)	1.00		1.00	
CT	430	46 (10.7)	0.68 (0.44–1.07)	0.092	0.59 (0.38–0.93)	0.024
CC	204	15 (7.4)	0.46 (0.25–0.84)	0.012	0.32 (0.17–0.60)	0.0004
Trend			0.68 (0.51–0.91)	0.009	0.57 (0.42–0.77)	0.0002
<i>DCPIA</i>						
rs11551405						
CC	530	47 (8.9)	1.00		1.00	
AC	287	39 (13.6)	1.60 (1.05–2.44)	0.030	2.02 (1.30–3.15)	1.80×10 <sup>-3</sup>
AA	41	9 (22.0)	2.97 (1.45–6.07)	0.003	2.23 (1.03–4.81)	0.041
Trend			1.67 (1.22–2.29)	0.001	1.66 (1.21–2.27)	1.50×10 <sup>-3</sup>

SNP, single nucleotide polymorphisms; CM, cutaneous melanoma; HR, hazards ratio; DSS, disease-specific survival; HR, hazards ratio; 95% CI, 95% confidence interval;

\* Adjusted by age, sex, tumor stage, Breslow thickness, ulceration of tumor, tumor cell mitotic rate in the Cox models, in additive model.



Table 3

Meta-analysis of three tagSNPs of the PIWI-piRNA pathway for the effects on CM DSS in an additive model

SNP	Gene	MDACC cohort (Discovery)			Harvard cohort (Replication)			Joint analysis		Heterogeneity test	
		HR (95% CI) +	P <sup>+</sup>	HR (95% CI) *	P <sup>✱</sup>	HR (95% CI) +	P <sup>+</sup>	HR (95% CI)	P*	I <sup>2</sup>	P <sub>het</sub>
DSS											
rs11551405	<i>DCPIA</i>	1.64 (1.21–2.23)	0.003	1.66 (1.21–2.27)	1.50×10 <sup>-3</sup>	1.55 (1.03–2.34)	0.038	1.62 (1.26–2.08)	1.55×10 <sup>-4</sup>		0.792
rs7933369	<i>PIWIL4</i>	1.57 (1.18–2.09)	0.002	1.97 (1.45–2.67)	1.43×10 <sup>-5</sup>	0.95 (0.66–1.36)	0.767	1.37 (0.67–2.82)	0.393		0.002
rs508485	<i>PIWIL4</i>	0.66 (0.50–0.89)	0.006	0.57 (0.42–0.77)	0.0002	1.05 (0.73–1.50)	0.806	0.77 (0.42–1.39)	0.386		0.011
Competing risk model											
rs11551405	<i>DCPIA</i>	1.63 (1.20–2.21)	0.002	1.61 (1.15–2.26)	0.006	1.53 (1.03–2.25)	0.033	1.58 (1.22–2.03)	3.47×10 <sup>-4</sup>		0.847
rs7933369	<i>PIWIL4</i>	1.58 (1.17–2.14)	0.003	1.99 (1.45–2.74)	2.50×10 <sup>-5</sup>	0.93 (0.65–1.35)	0.714	1.37 (0.65–2.88)	0.406		0.002
rs508485	<i>PIWIL4</i>	0.66 (0.49–0.88)	0.005	0.56 (0.41–0.76)	0.0002	1.06 (0.74–1.52)	0.742	0.77 (0.41–1.43)	0.408		0.008

HR, hazards ratio; 95% CI, 95% confidence interval; DSS, disease specific survival.

\* Adjusted by age, sex, tumor stage, Breslow thickness, ulceration of tumor, tumor cell mitotic rate in the Cox models;

✱ If  $P_{het} < 0.05$ , meta-analysis was performed with fixed effect model, if  $P_{het} \geq 0.05$ , meta-analysis was performed using random effects model.

<sup>+</sup> Adjusted by age, sex.