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Research Article

Association of Inflammation-related Genetic Variants with Melanoma Development and Progression

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Abstract

The role of inflammation in melanoma development and progression has not been fully elucidated. This study was aimed at determining whether previously identified inflammation-related genetic variants are associated with melanoma risk, clinical and pathologic measures of disease severity, or patient outcome, and might therefore contribute to the risk of development or progression of melanoma. We analyzed 11 single-nucleotide polymorphisms (SNPs) associated with inflammatory biomarkers that reached genome-wide significance of $P<10^{-8}$ in previously reported studies. All SNPs included in the analysis were genotyped or imputed in The University of Texas MD Anderson Cancer Center melanoma case-control melanoma dataset. One SNP, rs3845624 A allele, was associated with melanoma susceptibility (odds ratio=1.14, 95% confidence interval [CI] 1.02-1.27, *P*=0.019) but conversely with protection against melanoma progression (overall survival [OS] hazard ratio [HR]=0.87, 95% CI 0.77-0.99, *P*=0.033; melanoma-specific survival [MSS] HR=0.87, 95% CI 0.76-1.00, *P*=0.059). These effects remained significant after covariate adjustment. A second SNP, rs12034598 A allele, was associated with protection against both melanoma susceptibility (odds ratio=0.82, 95% CI 0.71-0.95, *P*=0.008) and melanoma progression (OS HR=0.77, 95% CI 0.64-0.93, *P*=0.006; MSS HR=0.75, 95% CI 0.60-0.92, *P*=0.007). However, the association with protection against melanoma progression was not significant after covariate adjustment. None of the SNPs were significantly associated with melanoma risk or progression after adjustment for multiple testing. Our study suggests that previously identified inflammatory biomarker related genetic variants may play a role in melanoma development or progression; some variants may have paradoxical associations. Association of these genetic variants with melanoma risk, disease severity and patient outcomes requires further investigation.

Keywords: Inflammatory Biomarker; Common SNPs; Melanoma Risk; Melanoma Progression

Introduction

Melanoma is the major cause of death from skin cancer; advanced melanoma is considered one of the most therapy-resistant malignancies [1]. The incidence of melanoma continues to increase in the United States [2,3]. High-penetrance genetic variants associated with melanoma risk, such as cyclin-dependent kinase inhibitor 2A (CDKN2A), cyclin-dependent kinase 4 (CDK4), and a locus on 1p22 [4,5], have been identified in patients with familial aggregation of melanoma but account for only a small proportion of variation in melanoma susceptibility because of their low frequency in the general population. Gene association studies (GWASs) have identified several common genetic variants that demonstrate low to medium effect on pigmentation or nevi and melanoma risk [6-17].

Prevalence studies have demonstrated an association between inflammatory biomarkers and colorectal or lung cancer, but prospective studies have failed to provide consistent evidence for a biological role in cancer [18,19]. Studies have shown that chronic inflammation may create a microenvironment that promotes tumor growth [18-20]. Inflammatory biomarkers including blood levels of C-reactive protein (CRP) have been associated with poor prognosis in breast, lung, and several other cancers [21-25], including melanoma [26]. To further elucidate the potential biological mechanisms of the inflammatory process in melanoma development and progression, we investigated whether previously identified inflammatory biomarker related genetic polymorphisms might be associated with melanoma risk, clinical and pathologic measures of disease severity, or melanoma progression.

Materials and Methods

Study Design

A hospital-based, case-control investigation of cutaneous melanoma was conducted at The University of Texas MD Anderson Cancer Center between March 1998 and August 2008. A total of 3,156 non-Hispanic white patients and controls were recruited. After genotyping samples from all participants, we determined that 1,804 melanoma patients and 1,026 cancer-free controls (friends or acquaintances of patients reporting to other clinics) had adequate single-nucleotide polymorphism (SNP) data for analysis. The study population was described previously [27]. All individuals provided written informed consent to participate in this study under a protocol approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center.

Patients with all stages of cutaneous melanoma evaluated in the Melanoma and Skin Center at MD Anderson were eligible for inclusion. Data on sex, age, and clinical prognostic factors were collected from patient records. Clinical prognostic factors collected included 2009 American Joint Committee on Cancer stage[28], Breslow tumor thickness, ulceration, mitosis, and sentinel lymph node status. Length of follow-up and survival duration were determined from the date of diagnosis until last contact or the date or death, respectively. A total of 1,800 patients had follow-up data available. Patients were categorized as having a melanoma recurrence if they developed local, regional, in-transit, or distant metastasis during the follow-up period.

Genotyping and Data Quality Control

High-density genotype data were obtained from DNA samples of all 3,156 participants. The samples were genotyped by using the Illumina HumanQmnil-Quad_v1-0_B array with data quality control as described previously[27]. A total of 818,237 genotyped SNPs were selected for the primary analysis. Imputation of ungenotyped SNPs was performed using the MACH [29] program with genotype data from all subjects. In total, 2,649,586 imputed or directly genotyped SNPs eligible for an association study. From those candidates, we selected 11 SNPs listed in the National Human Genome Research Institute GWAS Catalog[30] as being associated with inflammatory biomarkers such as IL18, IL-1ra, IL-6, ESR, MCP, CRP, TNF at a genome-wide level of significance ($P<5\times10^{-8}$) (Table 1, Supplementary Table 1).

Statistical Analysis

The SNPs and their associations with inflammatory biomarkers from previous studies are summarized in Supplementary Table 1. We chose 11 SNPs with *P* values less than the nominal GWAS significance level for biomarkers and analyzed the associations of the SNPs with melanoma susceptibility, progression, and outcome (stage, ulceration, tumor thickness, mitosis, recurrence, and death).

We first performed logistic regression modeling to measure the additive effect of each individual SNP on melanoma susceptibility, as well as on the standard melanoma clinical and pathologic measures of disease severity of stage (III-IV vs I-II), primary tumor ulceration (present vs absent), and primary tumor mitosis ($\geq1/mm^2$ vs $<1/mm^2$), without adjustment for any covariates. We also performed linear regression analysis of the genetic effect of each SNP on log-transformed primary tumor thickness, another standard measure of melanoma disease severity. We finally evaluated the relationship between each SNP and important patient outcome measures, including overall survival (OS), melanoma-specific survival (MSS), and disease-free survival (DFS), by Cox regression in SAS Enterprise Guide 4.3 (SAS Institute, Cary, NC)[31]. Sex, age, and primary tumor thickness were adjusted in the multivariate analysis. A *P* value of 0.05 was considered nominally significant. Corrections for multiple testing were made using Bonferroni adjustments.

Results

Demographic Data for Melanoma Patients and Controls

Totally 2,829 observations from MD Anderson were included in the analysis (one individual with missing age information was excluded). For the 1,804 melanoma patients, mean age at diagnosis was 52.1 years; 21.6% of the patients had stage III/IV disease at diagnosis, 60.4% had primary tumor thickness greater than 1 mm, 18.5% had ulcerated primary tumors, 64.0% had primary tumor mitotic rates greater than or equal to 1. Sentinel lymph node biopsy was executed in 917 of 1,412 patients (64.9%) who presented with clinically localized disease, including 641 of 771 patients (83.1%) with tumors at least 0.75 mm in thickness; 189 (17.0%) of

Table 1. SNPs listed in the National Human Genome Research Institute GWAS Catalog as being associated with inflammatory biomarkers.

SNP	Chromosome	Position	Reported gene(s)	*P* (association)		SNPs passing QC (imputed)
rs2250417	11	112214593	*BCO2, IL-18, TEX12*	2E-32	(*IL-18*)	2,543,887 (Illumina)
rs7577696	2	32053713	*SRD5A2, DPY30, SPAST, SLC30A6, NLRC4*	3E-19	*(IL-18)*	Illumina [2,543,887] (imputed)
rs6743376	2	113074756	*IL1F10*	2E-26	*(IL-1ra)*	Illumina [2,543,887] (imputed)
rs643434	9	133266942	*ABO*	9E-25	*(IL-6)*	Affymetrix [~1.9 million] (imputed)
rs12034598	1	207584170	*CR1*	9E-14	*(ESR)*	Affymetrix [~1.9 million] (imputed)
rs12075	1	159205564	*DARC*	4E-51	*(MCP-1)*	Affymetrix [~1.9 million] (imputed)
rs3026968	1	159177662	*CADM3*	9E-14	*(MCP-1)*	Affymetrix [~1.9 million] (imputed)
rs1341665	1	159721769	*CRP*	2E-20	*(hsCRP)*	Affymetrix [~1.9 million] (imputed)
rs3845624	1	159248476	*DARC*	2E-11	*(hsCRP)*	Affymetrix [~1.9 million] (imputed)
rs4910742	11	5285279	*HBB*	2E-9	*(ESR)*	Affymetrix [~1.9 million] (imputed)
rs7911500	10	5995763	*IL2RA*	5E-9	(hsCRP-IL6 pattern prefenofibrate)	Affymetrix [2,543,887](imputed)
rs12532960	7	42286026	Intergenic	3E-7	(hsCRP-IL6 pattern prefenofibrate)	Affymetrix [2,543,887](imputed)
rs6728440	2	19799585	Intergenic	2E-7	(hsCRP-IL6 pattern postfenofibrate)	Affymetrix [2,543,887](imputed)
rs3764563	19	15613392	*CYP4F8*	3E-7	(MCP1–TNF-α pattern prefenofibrate)	Affymetrix [2,543,887](imputed)
rs786870	10	26485765	*APBB1IP*	5E-7	(MCP1–TNF-α pattern prefenofibrate)	Affymetrix [2,543,887](imputed)
rs17564315	2	19758735	Intergenic	7E-7	(MCP1–TNF-α pattern prefenofibrate)	Affymetrix [2,543,887](imputed)
rs1396485	5	116176655	COMMD10	7E-7	(MCP1–TNF-α pattern prefenofibrate)	Affymetrix [2,543,887](imputed)
rs12722605	10	6011200	IL2RA	3E-7	(MCP1–TNF-α pattern postfenofibrate)	Affymetrix [2,543,887](imputed)
rs391317	11	36990987	Intergenic	5E-7	(MCP1–TNF-α pattern postfenofibrate)	Affymetrix [2,543,887](imputed)
rs13122273	4	13100727	Intergenic	9E-7	(CRP)	Affymetrix [2,543,887](imputed)
rs17460823	14	33117357	NPAS3	2E-6	(CRP)	Affymetrix [2,543,887](imputed)
rs10888935	1	55595278	Intergenic	7E-7	(IL-6)	Affymetrix [2,543,887](imputed)
rs4513299	2	114292241	Intergenic	4E-6	(IL-6)	Affymetrix [2,543,887](imputed)
rs6517147	21	33170482	Intergenic	7E-7	(IL-2 soluble receptor α)	Affymetrix [2,543,887](imputed)
rs11661856	18	77941567	Intergenic	1E-6	(IL-2 soluble receptor α)	Affymetrix [2,543,887](imputed)
rs17556665	11	14076935	SPON1	1E-6	(TNF-α)	Affymetrix [2,543,887](imputed)
rs11979476	7	129353915	AHCYL2	2E-6	(TNF-α)	Affymetrix [2,543,887](imputed)
rs12220898	10	49368588	DRGX	1E-6	(MCP-1)	Affymetrix [2,543,887](imputed)
rs4909764	8	138243675	FAM135B	2E-6	(MCP-1)	Affymetrix [2,543,887](imputed)

QC, quality control; IL, interleukin; MCP, monocyte chemoattractant protein; hs, high sensitivity; TNF, tumor necrosis factor.

the sentinel lymph node biopsy samples were positive (Table 2; all $P < 0.0001$ with frequency compared across different groups).

SNP Association with Melanoma Risk and Clinical Factors

Among the 11 SNPs, rs3845624 (additive effect for allele A odds ratio 1.14, 95% confidence interval [CI] 1.02-1.27, *P*=0.019; Table 3) and rs12034598 (additive effect for allele A odds ratio 0.82, 95% CI 0.71-0.95, *P*=0.008; Table 3) were associated with melanoma risk at the nominal significance level of *P*<0.05 without adjustment for any covariate. SNP rs3845624 A allele also was associated with thicker tumor thickness (*P*=0.019; Table 3) at a nominal significance level of *P*<0.05. After Bonferroni correction for multiple testing of 11 SNPs, neither of these two SNPs remained significantly associated with melanoma susceptibility or tumor thickness. No SNPs were associated with tumor stage, ulceration, mitosis, or disease recurrence at the nominal significance level of *P*< 0.05 (Table 3).

Association between Each SNP and Melanoma Outcome

To determine whether inflammation-related genetic variants might contribute to patient outcomes (OS, MSS, DFS), we performed Cox regression analysis, with or without adjustment for age, sex, and tumor thickness. SNP rs3845624 was significantly associated with OS in univariate analysis (additive effect for allele A hazard ratio [HR]=0.87, 95% CI 0.77-0.99, *P*=0.033) and multivariate analysis with adjustment for sex, age, and tumor thickness (HR=0.81, 95% CI 0.67-0.98, *P*=0.030) (Table 4). This SNP was also associated at a borderline significance level with MSS in univariate analysis (HR=0.87, 95% CI 0.76-1.00, *P*=0.059) and significantly associated with MSS after adjustment for sex, age, and tumor thickness (HR=0.83, 95% CI 0.69-0.99, *P*=0.040). SNP rs12034598 was significantly associated with

Table 2. Clinical characteristics of the 1,804 patients.

Variable	N	Mean	*P-value**
Age at diagnosis, years		52.11 (SD 14.52)	-
Sex			
Male	1,059	41.3	<0.0001
Female	744	58.7	
Stage at diagnosis			
I-II	1,412	78.4	<0.0001
III-IV	388	21.6	
Tumor thickness, mm			
0-1	714	39.6	<0.0001
1-2	409	22.7	
2-4	269	14.9	
>4	154	8.6	
Ulceration			
Not present	1,164	81.5	<0.0001
Present	264	18.5	
Mitosis			
0-1/mm^2	417	36.0	<0.0001
>1/mm^2	742	64.0	
Sentinel lymph node biopsy among stage I-II			
Not conducted	495	35.1	<0.0001
Conducted	917	64.9	
Sentinel lymph node status			
Negative	926	83.0	<0.0001
Positive	189	17.0	

SD, standard deviation.
*P-value measures the difference of frequency distribution between each category of a covariate

Discussion

In this candidate gene association study, we analyzed 11 common inflammatory biomarker related SNPs previously identified in GWASs and found that two of the SNPs predicted melanoma risk and progression at the nominal significance level of $P<0.05$.

The association between the *DARC* gene (rs3845624) and melanoma risk had not been previously reported. Each additional copy of rs3845624 minor allele C was associated with an increase of 0.14 standard deviation in the level of high-sensitivity CRP in the original GWAS study[32]; therefore, presence of the major allele A would be predicted to result in a decrease in the level of high-sensitivity CRP. In this study, we observed an association of major allele A of this SNP with melanoma risk and progression. If the genetic effect of SNP rs3845624 on melanoma outcome is mediated by the intermediate inflammatory biomarker hsCRP, then one would predict based on our results that a low level of hsCRP should both increase melanoma susceptibility and prolong survival. The exact role of CRP in melanoma risk has not been determined, but a high level of CRP has been reported to contributes to poor survival among patients several cancers, including breast, lung, and other cancers[21-25], including melanoma[26].

Table 3. Association of inflammation-related genetic variants with melanoma risk and patient clinical features.

SNP	Allele	Allele frequency	Chr	Position	Association with melanoma susceptibility[a]		*P* for association with:				
					P	OR (95% CI)	Stage[b]	Log tumor thickness[c]	Ulceration[d]	Mitosis	DFS
rs3026968	C	0.7746	1	157414076	0.589	0.96 (0.85-1.10)	0.218	0.296	0.096	0.218	0.152
rs12075	A	0.584	1	157441978	0.924	0.99 (0.89-1.11)	0.343	0.960	0.434	0.343	0.803
rs3845624	A	0.5759	1	157484890	**0.019**	**1.14 (1.02-1.27)**	0.479	**0.019**	0.935	0.479	0.252
rs1341665	A	0.3376	1	157958183	0.106	0.91 (0.81-1.02)	0.653	0.202	0.301	0.653	0.242
rs12034598	A	0.8194	1	205824138	**0.008**	**0.82 (0.71-0.95)**	0.998	0.655	0.404	0.998	0.473
rs7577696	A	0.5966	2	32132286	0.788	0.98 (0.88-1.10)	0.901	0.951	0.786	0.901	0.003
rs6743376	A	0.6381	2	113548804	0.427	0.96 (0.85-1.07)	0.586	0.544	0.795	0.586	0.249
rs643434	A	0.3448	9	135132176	0.686	0.98 (0.87-1.09)	0.171	0.099	0.446	0.171	0.843
rs7911500	C	0.9263	10	6077732	0.323	1.14 (0.88-1.48)	0.235	0.841	0.503	0.235	0.773
rs4910742	A	0.9731	11	5263085	0.107	1.43 (0.93-2.19)	0.641	0.671	0.934	0.641	0.210
rs2250417	C	0.5382	11	111590526	0.310	0.94 (0.85-1.05)	0.675	0.420	0.529	0.675	0.825

OR, odds ratio; DFS, disease-free survival.
a. Samples from 2830 melanoma cases(=1804) and controls (=1026);
b. 388 patients with stage III/IV vs 1412 with stage I/II
c. 1547 patients with tumor thickness, log-transformed primary tumor thickness
d. Primary tumor ulceration present vs. absent
e. Mitosis >1/mm^2 vs 0-1/mm^2
f. Samples from 1412 stage I-II cases with relapse-free survival data (relapse=253);

OS (additive effect for allele A HR=0.77, 95% CI 0.64-0.93, P=0.006) and MSS (HR=0.75, 95% CI 0.60-0.92, P=0.007) in univariate analysis but was not associated with OS (HR=1.00, 95% CI 0.76-1.32, P=0.987) or MSS (HR=0.81, 95% CI 0.62-1.05, P=0.116) in multivariate analysis. Further stratification analysis showed that both SNPs were significantly associated with OS among patients with stage I-II disease (rs3845624 HR=0.84, 95% CI 0.72-0.98, P=0.028; rs12034598 HR=0.77, 95% CI 0.61-0.98, P=0.032) but not among patients with stage III-IV disease (Table 5). No association between any SNP and OS or MSS remained significant with or without adjustment for above covariates after Bonferroni adjustment for multiple comparisons.

SNP rs12034598, in the *CR1* gene region, was associated with a mean erythrocyte sedimentation rate (ESR) decrease of 1.024 mm/h per minor allele A in the original GWAS study[32]. In the present study, rs12034598 allele A was associated with decreased risk for melanoma susceptibility and longer patient survival. The role of the *CR1* gene or ESR in melanoma susceptibility has rarely been reported; interestingly, an ESR greater than 15 mm/h has been reported to be associated with short survival in patients with metastatic malignant melanoma[33]. The mechanism by which the *CR1* gene might contribute to melanoma progression is not understood. High blood levels of CRP increase the ESR, and high CRP and high ESR are used in

Table 4. Association of inflammation-related genetic variants with melanoma survival.

SNP	Allele	Allele frequency	Chr	Position	Overall survival				Melanoma-specific survival			
					Univariate analysis		Multivariate analysis*		Univariate analysis		Multivariate analysis*	
					HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
rs3026968	C	0.7746	1	157414076	**0.84 (0.71-0.99)**	**0.040**	1.03 (0.79-1.33)	0.835	0.84 (0.69-1.02)	0.080	1.01 (0.78-1.30)	0.948
rs12075	A	0.584	1	157441978	1.03 (0.91-1.17)	0.620	1.08 (0.89-1.30)	0.451	1.01 (0.87-1.17)	0.939	1.07 (0.89-1.30)	0.471
rs3845624	A	0.5759	1	157484890	**0.87 (0.77-0.99)**	**0.033**	**0.81 (0.67-0.98)**	**0.030**	0.87 (0.76-1.00)	0.059	**0.83 (0.69-0.99)**	**0.040**
rs1341665	A	0.3376	1	157958183	0.99 (0.89-1.09)	0.777	1.02 (0.87-1.19)	0.835	0.99 (0.89-1.12)	0.927	1.08 (0.93-1.26)	0.293
rs12034598	A	0.8194	1	205824138	**0.77 (0.64-0.93)**	**0.006**	1.00 (0.76-1.32)	0.987	**0.75 (0.60-0.92)**	**0.007**	0.81 (0.62-1.05)	0.116
rs7577696	A	0.5966	2	32132286	1.06 (0.93-1.21)	0.360	0.98 (0.80-1.21)	0.872	1.08 (0.93-1.26)	0.311	1.06 (0.87-1.29)	0.577
rs6743376	A	0.6381	2	113548804	1.02 (0.89-1.17)	0.793	1.11 (0.90-1.37)	0.328	1.11 (0.94-1.30)	0.208	1.10 (0.90-1.35)	0.355
rs643434	A	0.3448	9	135132176	0.95 (0.86-1.06)	0.369	0.90 (0.77-1.05)	0.181	0.96 (0.86-1.08)	0.503	0.91 (0.79-1.06)	0.238
rs7911500	C	0.9263	10	6077732	1.06 (0.74-1.52)	0.746	1.43 (0.86-2.37)	0.184	1.09 (0.73-1.65)	0.674	1.00 (0.60-1.67)	0.997
rs4910742	A	0.9731	11	5263085	1.17 (0.66-2.04)	0.599	0.92 (0.36-2.34)	0.862	1.24 (0.65-2.34)	0.520	**2.14 (1.09-4.20)**	**0.040**
rs2250417	C	0.5382	11	111590526	1.00 (0.89-1.13)	0.939	1.00 (0.84-1.20)	0.968	0.97 (0.85-1.11)	0.658	0.95 (0.79-1.13)	0.533

*Adjusted for sex, age, and tumor thickness. Samples from 1801 cases with overall survival data (death=260);

Table 5. Association of inflammation related genetic variants with overall survival among patients in different stages.

SNP	Allele	Allele frequency	Chr	Position	Stage I-II[a]		Stage III-IV[b]	
					HR (95% CI)	*P*	HR (95% CI)	*P*
rs3026968	C	0.7746	1	157414076	0.78 (0.63-0.97)	0.024	0.96 (0.73-1.25)	0.750
rs12075	A	0.584	1	157441978	1.02 (0.86-1.20)	0.830	1.06 (0.86-1.31)	0.578
rs3845624	A	0.5759	1	157484890	**0.84 (0.72-0.98)**	**0.028**	0.94 (0.77-1.16)	0.579
rs1341665	A	0.3376	1	157958183	0.97 (0.85-1.11)	0.680	1.07 (0.91-1.26)	0.401
rs12034598	A	0.8194	1	205824138	**0.77 (0.61-0.98)**	**0.032**	0.89 (0.66-1.21)	0.461
rs7577696	A	0.5966	2	32132286	1.12 (0.94-1.32)	0.204	0.98 (0.79-1.20)	0.813
rs6743376	A	0.6381	2	113548804	1.01 (0.85-1.21)	0.903	1.03 (0.84-1.27)	0.774
rs643434	A	0.3448	9	135132176	0.97 (0.85-1.10)	0.599	0.91 (0.78-1.07)	0.262
rs7911500	C	0.9263	10	6077732	1.12 (0.71-1.76)	0.629	1.01 (0.57-1.80)	0.964
rs4910742	A	0.9731	11	5263085	1.40 (0.71-2.73)	0.343	1.16 (0.40-3.36)	0.782
rs2250417	C	0.5382	11	111590526	1.03 (0.89-1.20)	0.670	0.95 (0.79-1.15)	0.616

a. Samples from 1412 stage I-II cases with overall survival data (death=150);
b. Samples from 388 stage III-IV cases with overall survival data (death=110);

clinical practice as nonspecific inflammatory biomarkers. Some SNP alleles are associated with increased levels of both CRP and the ESR[32]. Therefore, the *CR1* gene might help control both blood CRP levels and the ESR, and one or both of these inflammatory biomarkers might contribute to melanoma progression.

Our study had limitations and therefore should be considered preliminary. First, the significance of the association between genetic variants and melanoma susceptibility and progression was not confirmed after Bonferroni adjustment for multiple testing. Second, our results were based only on data from patients at MD Anderson and were not externally validated. Therefore, analysis of an independent validation data set is needed to confirm our findings. Third, our analysis did not incorporate the gene-gene and gene-environment interactions that are common in complex diseases such as cancer[34]. Fourth, our risk prediction model was based

exclusively on data from non-Hispanic whites. We assumed there was no population substructure and therefore did not adjust our analysis for ethnicity.

In summary, our preliminary results suggest that previously identified inflammatory-biomarker-associated genetic variants may play a role in melanoma development and progression. The association between these genetic variants and patient outcomes needs to be investigated further. Future studies should also investigate the effects of gene-gene and gene-environment interactions as well as epigenetic markers.

Conflict of Interest statement

None declared.

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