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Inherited variation at *MC1R* and *ASIP* and association with melanoma-specific survival

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This investigation of germline variation at MC1R, as well as risk haplotypes near the ASIP locus, and survival in a large population-based series of incident single primary melanomas reports evidence of improved melanoma-specific survival among carriers of more than one MC1R variant. We also demonstrate an increased hazard of melanoma-specific death among carriers of the TG/TG ASIP diplotype. These results support the influential role that pigmentary genetic loci play on melanoma outcomes.

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Author Contributions

NJT and PAK designed the analytic question, interpreted the analysis of data, and prepared the manuscript.
AR, NJT and PAK performed the analysis of data.
PAK and TR performed all *MC1R* genotyping.
IO performed all *ASIP* genotyping.
KAW performed Illumina genotyping.
MB and CB conceived and designed the GEM Study and also contributed to data collection and critical review of the manuscript.
All other authors (KJB, LF, PAG, HAC, AEC, TD, RPG, SG, IO, SR, NET, RZ, TRR) contributed to data collection and critical review of the manuscript.

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Abstract

MC1R is a marker of melanoma risk in populations of European ancestry. However, *MC1R* effects on survival are much less studied. We investigated associations between variation at *MC1R* and survival in an international, population-based series of single primary melanoma patients enrolled into the GEM study. *MC1R* genotype data was available for 2,200 participants with a first incident primary melanoma diagnosis. We estimated the association of *MC1R* genotypes with melanoma-specific survival (*i.e.* death due to melanoma) and overall survival using Cox proportional hazards modeling, adjusting for established prognostic factors for melanoma. We also conducted stratified analyses by Breslow thickness, tumor site, phenotypic index and age. Additionally, we evaluated haplotypes involving polymorphisms near the *ASIP* locus for their impacts on survival. Melanoma-specific survival was inversely associated with carriage of *MC1R* variants in the absence of consensus alleles compared to carriage of at least one consensus allele (HR=0.60; 95%CI: 0.40, 0.90). *MC1R* results for overall survival were consistent with no association. We did not observe any statistical evidence of heterogeneity of effect estimates in stratified analyses. We observed increased hazard of melanoma-specific death among carriers of the risk haplotype TG near the *ASIP* locus (HR=1.37; 95%CI: 0.91, 2.04) when compared to carriers of the most common GG haplotype. Similar results were noted for overall survival. Upon examining the *ASIP* TG/TG diplotype, we observed considerably increased hazard of melanoma-specific death (HR=5.11; 95%CI: 1.88, 13.88) compared to carriers of the most common GG/GG diplotype. Our data suggest improved melanoma-specific survival among carriers of two inherited *MC1R* variants.

Introduction

Inherited variation at the melanocortin-1 receptor (*MC1R*) locus is an established marker of elevated melanoma risk in populations of European ancestry [1]. However, *MC1R* effects on survival are much less studied. *MC1R* has pigmentary and non-pigmentary biological functions [2, 3], both of which may be important for survival. Studies have shown that carriers of red hair color-associated (RHC) *MC1R* variants are at increased risk of melanoma [1] possibly due to diminished α-melanocortin mediation of DNA damage repair [4]. This reduced repair capacity combined with decreased eumelanin may render RHC variant carriers more susceptible to the deleterious effects of ultraviolet (UV) radiation [3]. Juxtaposed against increasing risk for melanoma, it has been suggested that *MC1R* variants confer less resistance to apoptosis and mitigate cell proliferation, thereby improving overall survival [5].

Other pigmentation genes associated with melanoma risk affect *MC1R* function, and may also impact survival. The *ASIP* locus of chromosome 20, which encodes the agouti signaling protein and acts as an antagonist of *MC1R* directed eumelanin synthesis, has been associated with cutaneous phenotype and melanoma risk [6–9]. In particular, genome-wide association studies demonstrated strong associations between haplotypes composed of polymorphisms near the *ASIP* locus and risk of melanoma [6, 10].

In this study, we evaluate variation at *MC1R* for associations with melanoma-specific survival (*i.e.* death due to melanoma) and overall survival in a large population-based study of melanoma–The Genes, Environment, and Melanoma (GEM) Study. We also investigate the impact of a risk haplotype comprising alleles of rs4911414 and rs1015362, which lie ~110kb upstream of the *ASIP* locus, on survival. The GEM Study includes individuals with a diagnosis of first incident primary invasive melanoma (SPM) recruited from eight population-based cancer registries and one hospital-based study in Australia, Canada, Italy, and the United States for whom the entire coding region of *MC1R* was sequenced and two single nucleotide polymorphisms (SNPs) near the *ASIP* locus were genotyped.

Methods

GEM Study

The GEM Study is a population-based case-control study that enrolled a large series of individuals diagnosed with a SPM (n=2,424), in addition to 1,206 individuals with an incident second or higher order melanoma (MPM). We restrict our focus to SPM cases only due to previously reported melanoma risk differences between MPM and SPM with respect to *MC1R* [11]. Study participants were identified from eight population-based cancer registries and one hospital center in Australia, Canada, Italy and the United States. Details of GEM study methodology and procedures relating to study recruitment and survival outcomes have been previously described [12–15]. The local human research oversight committee approved standardized protocol procedures, and signed informed consent was obtained from all participants.

Diagnostic pathology reports were obtained for each participant from the appropriate ascertainment center, and data corresponding to histological subtype, lesion thickness, and anatomic location of lesion were abstracted. Tumor tissue slides for 2,105 (86.8%) participants were available for centralized pathological review, performed by one of three study pathologists. Standardized pathologic review of slides included evaluation of Breslow thickness and presence of ulceration. Since Breslow thickness was both abstracted from the pathology report and recorded during the centralized pathologic review, the measure corresponding to the deepest reading was chosen to represent the value of most biological relevance.

A phenotypic index was derived using data collected from a study participant self-administered questionnaire [16], and was based on: hair color (black or dark brown=1; light brown or blond=2; red=3), eye color (black or brown=0; all other colors=1), and relative inability to tan in response to sun exposure (no=0; yes=1) [11]. Phenotypic index scores of 1 and 2 indicate relatively darker cutaneous phenotypes and lower phenotypic melanoma risk;

an index score of 3 indicates medium phenotypic risk; and index scores of 4 and 5 indicate relatively fairer cutaneous phenotypes and higher phenotypic risks for melanoma.

MC1R and ASIP Genotyping

MC1R and *ASIP* genotypes were available for 2,200 (90.8%) participants, and we have previously reported on genotyping and prevalence of *MC1R* variants is this study sample [11]. We adopted nomenclature and definitions based on previous literature [1, 17–20] to classify *MC1R* variants as conferring higher risk for melanoma based on strong association with red hair phenotype [R] (D84E, R142H, R151C, R160W, and D294H, all nonsense and insertion/deletion) or lower risk for melanoma based on weaker association with red hair phenotype [r] (all other nonsynonymous variants). Since the exact functional status of many *MC1R* variants is still unknown, we acknowledge that these risk categories may be inaccurate. Based on a previous investigation of *MC1R* and overall survival from cutaneous melanoma [5], *MC1R* genotype was categorized in two ways to assess the relative impacts of *MC1R* variants and consensus (wild type) alleles on survival. Firstly, according to variant carriage number: carriage of only consensus alleles versus carriage of only one *MC1R* variant (high- [R] or low- [r] risk) versus carriage of two *MC1R* variants (high- [R] or low-[r] risk); and secondly, by carriage of at least one consensus allele versus carriage of two *MC1R* variants.

Two SNPs comprising a risk haplotype (rs4911414 and rs1015362) [21] located in the 5′-noncoding region ~110 kb upstream of the *ASIP* locus were genotyped; rs4911414 was genotyped using the Sequenom MassARRAY iPLEX genotyping platform (Sequenom Inc., San Diego, CA), with quality control measures implemented as previously described [22] and rs1015362 was genotyped as part of a larger panel of SNPs on a custom Illumina GoldenGate panel. To ensure quality control of Illumina data, assay intensity data and genotype cluster images were evaluated for rs1015362. *ASIP* haplotypes were constructed from participants' genotype data using the PHASE program v2.1 [23, 24] to infer haplotype probabilities when genotype data was missing or when genotype phase was ambiguous.

Statistical Analysis

The principal outcome of interest in this study was time from SPM diagnosis to death from melanoma, with secondary consideration for time from SPM diagnosis to death by any cause. We used Cox proportional hazards modeling via the SAS v9.3 (SAS Institute, Cary, NC) PHREG procedure to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between *MC1R* genotype or *ASIP* haplotype and melanoma-specific and overall survival, adjusting all models for age at diagnosis, sex, study center, natural log-transformed Breslow thickness, tumor site (categorized as head/neck, trunk/pelvis, arms, or legs), and ulceration. Because a small proportion (4%) of SPM were later ascertained and enrolled as a MPM, a dichotomous indicator variable was included in all models to account for potential bias introduced by these "crossover" participants.

To evaluate whether *MC1R* associations with survival outcomes were different across strata of selected host and tumor characteristics, we also modeled survival within levels of Breslow thickness, tumor site, age at diagnosis, and phenotypic index, and used the Wald

test to assess interaction terms. All statistical tests were two-sided with an alpha level of 0.05.

Results

Table 1 gives overall study characteristics and study characteristics according to survival outcomes for our GEM study population. Overall, there were 343 deaths among participants with SPM, of which 164 were attributed to melanoma. Median follow-up time was 7.6 years (interquartile range, 6.8–7.8).

We observed a statistically significant association between number of *MC1R* variants and hazard of melanoma-specific death (P_{trend}=0.04; Table 2). Compared to participants carrying only consensus *MC1R* alleles, there was a muted association among those carrying only a single variant (HR=1.13; 95%CI: 0.69, 1.87; Table 2) and an inverse association among participants carrying two variants (HR=0.65; 95%CI: 0.38, 1.13; Table 2). Based on these results, we categorized the *MC1R* variable according to carriage of two variants versus carriage of any consensus allele, noting a significant inverse association between dual variant carriage and hazard of melanoma-specific death (HR=0.60, 95%CI: 0.40, 0.90; Table 2). Associations of a six-level *MC1R* variable showing all combinations of r- and R-variants and melanoma-specific survival are given in Supplemental Table 1. Observed results for overall survival were closer to the null (Supplemental Tables 2 and 3). Utilizing the aforementioned dichotomous *MC1R* variable, we did not observe any statistical evidence of heterogeneity of effect estimates across these strata (Table 3); however, we did note isolated associations between *MC1R* and melanoma-specific death within strata of tumor site, age at first diagnosis, and phenotypic index. Results of stratified analyses for overall survival were similar to those for melanoma-specific survival (Supplemental Table 4).

Haplotype analysis of the *ASIP* locus suggested increased association between the risk haplotype TG--containing the minor allele of rs4911414 and the major allele of rs1015362--and melanoma-specific survival. Compared to carriage of the most common GG haplotype, carriage of TG was associated with poorer melanoma-specific survival (HR=1.37; 95%CI: 0.92, 2.05), although this association did not attain statistical significance (Table 2). We also analyzed *ASIP* diplotypes (combinations of two-SNP haplotypes) and observed that TG/TG was associated with a 5-fold increased hazard of melanoma-specific death when compared to carriage of GG/GG (HR=5.10; 95%CI: 1.88, 13.88) (Supplemental Table 5). We did not observe any statistical evidence of heterogeneity of haplotype effect estimates within strata of Breslow thickness, tumor site, age at first diagnosis, or phenotypic index (Table 3), and we saw no clear patterns of association.

Discussion

Results from the GEM Study indicate that melanoma-specific survival among individuals with SPM who are lacking a consensus *MC1R* allele is improved compared to those carrying at least one consensus allele. One previous study by Davies *et al.* [5] assessed the impact of inherited *MC1R* genotype on overall survival among melanoma patients. We directly recapitulated their published analysis, which required re-characterizing high and low risk

MC1R genotypes using a different classification scheme, recoding covariates, limiting the analysis set to GEM participants with thicker (>0.75mm) single primary melanomas, and limiting inference to overall survival. Using these filters, carriage of any *MC1R* variant in the absence of consensus alleles indicated better overall survival (HR=0.84; 95%CI: 0.64, 1.12; data not tabulated) versus carriage of at least one consensus allele in the GEM study. This hazard ratio estimate is similar to that of 0.78 (95%CI: 0.65, 0.94) previously reported. Although our result was not statistically significant, it is supportive of the meta-analytic finding by Davies *et al.* that was based on a total of 3,060 melanoma cases.

It is an interesting paradox that inherited variation at *MC1R* increases risk for development of melanoma, yet appears to provide a survival advantage to these same individuals. The association between *MC1R* variants and increased risk for melanoma is often discussed in terms of decreased eumelanogenesis and the concomitant reduction in protection against the known deleterious effects of UV radiation. A plausible alternative mechanism augmenting risk among *MC1R* variant carriers, yet also conferring a survival benefit involves pheomelanin synthesis. *MC1R* variant carriage is associated with higher pheomelanin-to-eumelanin ratios [25]; and murine models have suggested an integral role of pheomelanin in normal cell survival and growth by regulating rates of cystine delivery into cells, promoting defense against reactive oxygen species [26].

We observed a non-statistically significant modest increased hazard of melanoma-specific death among individuals with SPM carrying the TG haplotype composed of alleles from SNPs rs4911414 and rs1015362 near the *ASIP* locus. The risk haplotype has been previously reported by Maccioni *et al.* [21], who noted increased risk of melanoma among carriers (OR=1.91; 95%CI: 1.42, 2.57). Assessing the impact of carriage of at least one dual TG diplotype carriage on risk of melanoma, they noted a stronger association (OR=2.30; 95%CI: 1.58, 3.33). Our finding that the *ASIP* TG/TG diplotype is associated with a 5-fold increased hazard of death due to melanoma (HR=5.10; 95%CI: 1.88, 13.85) is consistent with the risk finding of Maccioni *et al*., although we recognize that our HR estimate is based on small numbers and is consistent with a smaller effect size. The TG haplotype has also been positively associated with fair skin color among Caucasians in the Nurses' Health Study [8], and positively associated with red hair color, freckling, and propensity for sunburn among Europeans [27]. Although we did not observe any statistically significant difference in effect estimates by phenotypic index category, we did notice increasing hazard ratios with increasing level of phenotypic index among carriers of TG and a notably strong association among the highest index category (Table 3). It is important to note that the SNPs comprising the haplotype under investigation do not map to *ASIP* and are in high LD with a large number of variants that map to other biologically plausible loci [21].

The GEM Study relied on death certificate information to verify cause of death, and misclassification is a potential concern [28]. Although GEM Study centers made every effort to ensure accurate ascertainment of participant deaths and causes, it is possible that variation in site reporting of death certificate information could have biased our data. While GEM benefitted from a robust follow-up time (~7.6 years), we also acknowledge the possibility that some melanoma attributed deaths occurred after follow-up ended and were not identified.

The GEM Study is uniquely positioned to investigate the relationship between *MC1R* and survival. The population-based design is a strength in that melanoma survival is likely representative of what we might expect in populations of European ancestry. However, the population-based nature of GEM also implies that a preponderance of thin or very early stage melanomas will be captured (~84% based on SEER data [29]), and effects on survival might be more easily elucidated in patients with more advanced lesions who are at higher risk of melanoma death. We previously examined melanoma tumor characteristics among SPM GEM Study participants, and found no evidence to suggest an association between AJCC established prognostic factors and *MC1R* variants (data not published).

In summary, our findings suggest that carriage of *MC1R* variants in the absence of consensus alleles is associated with better melanoma-specific survival among individuals with a first incident primary melanoma. In contrast, carriage of the risk haplotype TG near the *ASIP* locus was associated with poor melanoma-specific survival among those same individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of Abbreviations

ASIP	agouti signaling protein gene
CI	confidence interval

GEM	Genes, Environment and Melanoma Study
HR	hazard ratio
MC1R	melanocortin-1 receptor gene
MPM	second or higher order incident melanoma
OR	odds ratio
[R]	high-risk *MC1R* variant
[r]	low-risk *MC1R* variant
SNP	single nucleotide polymorphism
SPM	first incident primary invasive melanoma
UV	ultraviolet

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Table 1

Study characteristics, melanoma-specific and overall survival among GEM Study single primary melanoma cases

		Melanoma-specific	Overall
	N (%[a])	Deaths (%[b])	Deaths (%[b])
Age			
<40	488 (*20*)	16 (*3*)	20 (*4*)
40–54	735 (*30*)	39 (*5*)	52 (*7*)
55–64	451 (*18*)	35 (*8*)	51 (*11*)
≥65	794 (*32*)	74 (*9*)	220 (*28*)
Sex			
Male	1278 (*52*)	112 (*9*)	240 (*19*)
Female	1190 (*48*)	52 (*4*)	103 (*9*)
Center			
British Columbia, Can.	118 (*5*)	7 (*6*)	15 (*13*)
California, USA	219 (*9*)	9 (*4*)	19 (*9*)
Michigan, USA	318 (*13*)	20 (*6*)	35 (*11*)
New Jersey, USA	167 (*7*)	11 (*7*)	25 (*15*)
New South Wales, Aus.	725 (*29*)	60 (*9*)	141 (*19*)
North Carolina, USA	285 (*12*)	10 (*4*)	29 (*10*)
Ontario, Can.	428 (*17*)	28 (*7*)	53 (*12*)
Tasmania, Aus.	81 (*3*)	6 (*7*)	9 (*11*)
Torino, Ita.	127 (*5*)	13 (*10*)	17 (*13*)
Body Site			
Trunk/Pelvis	1097 (*44*)	73 (*7*)	87 (*8*)
Head/Neck	382 (*15*)	45 (*12*)	159 (*42*)
Arms	460 (*19*)	21 (*5*)	53 (*12*)
Legs	529 (*21*)	25 (*5*)	44 (*8*)
Ulceration			
No	187 (*8*)	51 (*27*)	80 (*43*)
Yes	1826 (*74*)	87 (*5*)	214 (*12*)
missing	455 (*18*)	26 (*6*)	49 (*11*)
Breslow Depth			
0.01–1.00mm	1595 (*65*)	21 (*1*)	111 (*7*)
1.01–2.00mm	485 (*20*)	49 (*10*)	90 (*19*)
2.01–4.00mm	228 (*9*)	51 (*22*)	80 (*35*)
>4.00mm	126 (*5*)	42 (*33*)	58 (*46*)
missing	34 (*1*)	1 (*3*)	4 (*12*)
Multiple primaries			
No	2372 (*96*)	152 (*6*)	322 (*14*)
Yes	96 (*4*)	12 (*13*)	21 (*22*)

[a] Column percentages are presented

[b] Row percentages are presented

Table 2

Hazard ratios (HRs) and 95% confidence intervals (CIs) for associations between variation in *MC1R* and *ASIP* and melanoma-specific survival in GEM Study single primary melanoma cases

		Melanoma-specific Survival		
MC1R [a]		**N (% [b])**	**Deaths (% [c])**	**HR (95% CI)[d]**
	con/con	373 (*15*)	26 (*7*)	1.00
	r/con, R/con	984 (*40*)	65 (*7*)	1.13 (0.69, 1.87)
	r/r, r/R, R/R	843 (*34*)	47 (*6*)	0.65 (0.38, 1.13)
	Missing	268 (*11*)	26 (*10*)	
				P_{trend}=0.04
	con/con, r/con, R/con	1357 (*55*)	91 (*7*)	1.00
	r/r, r/R, R/R	843 (*34*)	47 (*6*)	0.60 (0.40, 0.90)
	Missing	268 (*11*)	26 (*10*)	
				P=0.01
***ASIP* haplotypes**[e]				
	GG	2927 (*57*)	211 (*7*)	1.00
	TA	1280 (*24*)	69 (*5*)	0.83 (0.60, 1.14)
	TG	524 (*12*)	37 (*7*)	1.37 (0.92, 2.05)
	GA	205 (*6*)	11 (*5*)	1.19 (0.60, 2.38)

[a] Con=consensus, r=any low risk variant, R=any high risk variant.

[b] Column percentages are reported

[c] Row percentages are reported

[d] Adjusted for age at diagnosis, sex, center, natural log-transformed continuous Breslow thickness, tumor site, ulceration, and multiple melanomas.

[e] *ASIP* haplotype counts represent twice the number of individuals and are estimated by PHASE software assigning the most likely haplotype to an individual. HR's and 95% CI's were calculated using haplotype probabilities produced by PHASE, and were modeled in the PHREG procedure of SAS v9.3 (SAS Institute, Cary, NC). Haplotypes are inferred from SNPs rs4911414 (G/T) and rs1015362 (G/A).

Table 3

MC1R, ASIP haplotypes, and melanoma-specific survival in GEM single primary melanoma cases by strata of: Breslow thickness, tumor site, age, and phenotypic index

	N (%[a])	Deaths (%[b])	HR[c] (95% CI)	N (%[a])	Deaths (%[b])	HR[c] (95% CI)	N (%[a])	Deaths (%[b])	HR[c] (95% CI)	N (%[a])	Deaths (%[b])	HR[c] (95% CI)	P_{Het}
Breslow Thickness													
	0.01–1.00 mm			1.01–2.00 mm			2.01–4.00 mm			>4.00 mm			
MC1R [d]													
con/con, r/con, R/con	888 (*56*)	9 (*1*)	1.00	266 (*55*)	26 (*10*)	1.00	112 (*49*)	30 (*27*)	1.00	68 (*54*)	25 (*37*)	1.00	
r/r, R/r, R/R	538 (*34*)	5 (*<1*)	0.57 (0.14, 2.27)	167 (*34*)	16 (*10*)	0.85 (0.40, 1.83)	87 (*38*)	15 (*17*)	0.42 (0.20, 0.86)	40 (*32*)	11 (*28*)	0.43 (0.16, 1.11)	0.56
missing	169 (*11*)	7 (*4*)		52 (*11*)	7 (*13*)		29 (*13*)	6 (*21*)		18 (*14*)	6 (*33*)		
***ASIP H* aplotype**[e]													
GG	1440 (*45*)	8 (*<1*)	1.00	440 (*45*)	48 (*11*)	1.00	208 (*46*)	52 (*25*)	1.00	125 (*50*)	45 (*36*)	1.00	
TA	623 (*20*)	5 (*<1*)	0.90 (0.36, 2.24)	202 (*21*)	16 (*8*)	0.80 (0.43, 1.48)	79 (*17*)	12 (*15*)	0.58 (0.31, 1.07)	46 (*18*)	14 (*30*)	1.22 (0.57, 2.60)	0.64
TG	300 (*9*)	3 (*1*)	1.13 (0.35, 3.67)	75 (*8*)	9 (*12*)	1.03 (0.45, 2.35)	47 (*10*)	13 (*28*)	1.52 (0.82, 2.81)	23 (*9*)	6 (*26*)	1.64 (0.62, 4.29)	
GA	141 (*4*)	2 (*1*)	2.00 (0.41, 9.64)	39 (*4*)	1 (*3*)	0.32 (0.04, 2.27)	16 (*4*)	5 (*31*)	0.90 (0.26, 3.16)	8 (*3*)	3 (*38*)	8.82 (1.86, 41.93)	
missing	686 (*22*)	18 (*3*)		214 (*22*)	24 (*11*)		106 (*23*)	20 (*19*)		50 (*20*)	16 (*32*)		
Tumor Site													
	Head/Neck			Trunk/Pelvis			Arms			Legs			
MC1R[d]													
con/con, r/con, R/con	215 (*56*)	20 (*9*)	1.00	614 (*55*)	44 (*7*)	1.00	237 (*52*)	13 (*5*)	1.00	291 (*55*)	14 (*5*)	1.00	
r/r, R/r, R/R	131 (*34*)	18 (*14*)	0.87 (0.39, 1.93)	359 (*33*)	16 (*4*)	0.38 (0.19, 0.78)	171 (*37*)	5 (*3*)	0.49 (0.14, 1.67)	182 (*34*)	8 (*4*)	0.71 (0.24, 2.07)	0.88
missing	36 (*9*)	7 (*19*)		124 (*11*)	13 (*10*)		52 (*11*)	3 (*6*)		56 (*11*)	3 (*5*)		
***ASIP H* aplotype**[e]													
GG	337 (*44*)	47 (*14*)	1.00	978 (*45*)	68 (*7*)	1.00	424 (*46*)	20 (*5*)	1.00	509 (*48*)	11 (*2*)	1.00	
TA	146 (*19*)	15 (*10*)	1.00 (0.52, 1.92)	439 (*20*)	20 (*5*)	0.79 (0.49, 1.29)	177 (*19*)	8 (*5*)	0.83 (0.35, 1.94)	204 (*19*)	4 (*2*)	0.72 (0.30, 1.71)	0.65
TG	71 (*9*)	9 (*13*)	1.20 (0.52, 2.75)	209 (*10*)	15 (*7*)	1.36 (0.76, 2.44)	81 (*9*)	2 (*2*)	0.49 (0.11, 2.27)	90 (*9*)	5 (*6*)	2.76 (0.98, 7.73)	
GA	30 (*4*)	3 (*10*)	1.35 (0.32, 5.64)	92 (*4*)	5 (*5*)	0.96 (0.33, 2.79)	40 (*4*)	2 (*5*)	2.28 (0.40, 13.01)	43 (*4*)	1 (*2*)	3.02 (0.33, 27.49)	
missing	180 (*24*)	16 (*9*)		476 (*22*)	38 (*8*)		198 (*22*)	10 (*5*)		212 (*20*)	14 (*7*)		
Age													

	N (%[a])	Deaths (%[b])	HR[c] (95% CI)	N (%[a])	Deaths (%[b])	HR[c] (95% CI)	N (%[a])	Deaths (%[b])	HR[c] (95% CI)	N (%[a])	Deaths (%[b])	HR[c] (95% CI)	P[Het]
		<40			40–54			55–64			≥65		
MC1R [d]													
con/con, r/con, R/con	247 (*55*)	8 (*3*)	1.00	430 (*56*)	30 (*7*)	1.00	242 (*54*)	21 (*9*)	1.00	438 (*55*)	32 (*7*)	1.00	
r/r, R/r, R/R	172 (*38*)	5 (*3*)	0.75 (0.22, 2.56)	256 (*33*)	8 (*3*)	0.24 (0.09, 0.65)	164 (*36*)	9 (*5*)	0.47 (0.18, 1.25)	251 (*32*)	25 (*10*)	0.85 (0.45, 1.62)	0.22
missing	30 (*7*)	0		88 (*11*)	4 (*5*)		45 (*10*)	5 (*11*)		105 (*13*)	17 (*16*)		
***ASIP H* aplotype**[e]													
GG	432 (*48*)	16 (*4*)	1.00	700 (*45*)	42 (*6*)	1.00	420 (*47*)	30 (*7*)	1.00	696 (*44*)	73 (*10*)	1.00	
TA	182 (*20*)	1 (*<1*)	0.20 (0.03, 1.19)	323 (*21*)	18 (*6*)	0.96 (0.52, 1.80)	166 (*18*)	9 (*5*)	0.82 (0.37, 1.80)	295 (*19*)	19 (*6*)	0.92 (0.57, 1.50)	0.46
TG	88 (*10*)	5 (*6*)	4.10 (1.29, 12.96)	140 (*9*)	7 (*5*)	1.02 (0.42, 2.49)	77 (*9*)	7 (*9*)	1.30 (0.48, 3.50)	146 (*9*)	12 (*8*)	1.25 (0.67, 2.34)	
GA	36 (*4*)	0	no est.	73 (*5*)	5 (*7*)	1.91 (0.57, 6.38)	39 (*4*)	0	0.12 (0.005, 2.85)	57 (*4*)	6 (*11*)	2.28 (0.81, 6.39)	
missing	160 (*18*)	4 (*3*)		312 (*20*)	12 (*4*)		200 (*22*)	24 (*12*)		394 (*25*)	38 (*10*)		
					Phenotypic Index[f]								
		Low (1)			Medium (2, 3)			High (4, 5)					
MC1R [d]													
con/con, r/con, R/con	141 (*67*)	8 (*6*)	1.00	882 (*62*)	61 (*7*)	1.00	281 (*38*)	12 (*4*)	1.00				
r/r, R/r, R/R	52 (*25*)	7 (*13*)	1.49 (0.33, 6.74)	376 (*26*)	17 (*5*)	0.44 (0.22, 0.91)	371 (*51*)	18 (*5*)	0.91 (0.39, 2.13)				0.86
missing	19 (*9*)	0		166 (*12*)	22 (*13*)		19 (*11*)	4 (*5*)					
***ASIP H* aplotype**[e]													
GG	206 (*49*)	17 (*8*)	1.00	1324 (*46*)	99 (*7*)	1.00	634 (*43*)	28 (*4*)	1.00				
TA	85 (*20*)	6 (*7*)	0.78 (0.14, 4.26)	556 (*20*)	28 (*5*)	0.80 (0.53, 1.22)	280 (*19*)	6 (*2*)	0.83 (0.37, 1.82)				0.30
TG	26 (*6*)	1 (*4*)	0.20 (0.02, 2.48)	227 (*8*)	16 (*7*)	1.31 (0.75, 2.28)	174 (*12*)	8 (*5*)	1.61 (0.66, 3.94)				
GA	17 (*4*)	0	no est.	121 (*4*)	7 (*6*)	1.21 (0.51, 2.90)	56 (*4*)	4 (*7*)	2.89 (0.79, 10.62)				
missing	90 (*21*)	6 (*7*)		620 (*22*)	50 (*8*)		318 (*22*)	22 (*7*)					

[a] Column percentages are presented

[b] Row percentages are presented

[c] Hazard ratios (HR) and 95% confidence intervals (CI) adjusted for age at diagnosis (except age stratum), sex, study center, tumor site (except tumor site stratum), ulceration, natural log-transformed continuous Breslow thickness (except Breslow thickness stratum), and multiple melanomas.

[d] Con=consensus, r=any low risk variant, R=any high risk variant.

[e] *ASIP* haplotype counts represent twice the number of individuals and are estimated by PHASE software assigning the most likely haplotype to an individual. HR's and 95% CI's were calculated using haplotype probabilities produced by PHASE, and were modeled in the PHREG procedure of SAS v9.3 (SAS Institute, Cary, NC). Haplotypes are inferred from SNPs rs4911414 (G/T) and rs1015362 (G/A).

[f] Phenotypic index is based on pigmentation traits associated with a sun sensitive phenotype (e.g. lighter/red hair, lighter eyes, and a relative inability to tan), with higher scores associated with increasing sun sensitive phenotypes and lower scores associated with more sun resistant phenotypes (e.g. darker hair, darker eyes, and a relative ability to tan).

P_{Het} = P for heterogeneity