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Article

Association of functional, inherited vitamin D–binding protein variants with melanoma-specific death

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Abstract

Background: It is unclear whether genetic variants affecting vitamin D metabolism are associated with melanoma prognosis. Two functional missense variants in the vitamin D–binding protein gene (*GC*), rs7041 and rs4588, determine 3 common haplotypes, Gc1s, Gc1f, and Gc2, of which Gc1f may be associated with decreased all-cause death among melanoma patients based on results of a prior study, but the association of Gc1f with melanoma-specific death is unclear.

Methods: We investigated the association of the Gc1s, Gc1f, and Gc2 haplotypes with melanoma-specific and all-cause death among 4490 individuals with incident, invasive primary melanoma in 2 population-based studies using multivariable Cox-proportional hazards regression.

Results: In the pooled analysis of both datasets, the patients with the Gc1f haplotype had a 37% lower risk of melanoma-specific death than the patients without Gc1f (hazard ratio [HR] = 0.63, 95% confidence interval [CI] = 0.47 to 0.83, $P = .001$), with adjustments for age, sex, study center, first- or higher-order primary melanoma, tumor site, pigmentary phenotypes, and Breslow thickness. Associations were similar in both studies. In pooled analyses stratified by Breslow thickness, the corresponding melanoma-specific death HRs for those patients with the Gc1f haplotype compared with those without Gc1f were 0.89 (95% CI = 0.63 to 1.27) among participants with tumor Breslow thickness equal to or less than 2.0 mm and 0.40 (95% CI = 0.25 to 0.63) among participants with tumor Breslow thickness greater than 2.0 mm ($P_{interaction} = .003$).

Conclusions: Our findings suggest that individuals with the *GC* haplotype Gc1f may have a lower risk of dying from melanoma—specifically from thicker, higher-risk melanoma—than individuals without this Gc1f haplotype.

Vitamin D may regulate several pathways involved in cancer progression, including cell proliferation, apoptosis, angiogenesis, and metastasis, through activation of the vitamin D receptor (VDR) (1). Higher 25-hydroxyvitamin D_3 concentrations—the primary circulating form of vitamin D, which is used clinically to assess vitamin D status—as well as VDR variants and higher VDR expression may be associated with lower melanoma stage and better survival outcomes (2-6). However, it is unclear whether variants in other vitamin D genes, such as the vitamin D–binding protein (DBP) gene (*GC*), influence melanoma prognosis.

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Nearly 90% of circulating 25-hydroxyvitamin D_3 is bound to DBP, which can affect vitamin D half-life and bioavailability to target tissues (7) and can also be converted into a macrophage-activating factor (GcMAF), which has been shown to stimulate macrophage phagocytosis and inhibit tumor growth in mice and some cancer cell lines (8,9). Vitamin D concentrations and GcMAF activity may differ by the inherited *GC* haplotypes Gc1s, Gc1f, and Gc2 (also known as DBP1s, DBP1f, and DBP2) encoded by 2 *GC* missense variants altering the amino acid sequence at positions 432 and 436: rs7041 (g.57904T>G p. Asp432Glu) and rs4588 (g.57915C>A p. Thr436Lys) (see "Methods" for complete reference sequences) (10). The amino acids unique to each rs7041+rs4588 haplotype are as follows: Gc1s (p.432Glu+p.436Thr), Gc1f (p.432Asp+p.436Thr), and Gc2 (p.432Asp+p.436Lys).

In a prior epidemiological study of 9 melanoma cohorts (BioGenoMEL consortium), the Gc1f haplotype, relative to Gc2 or Gc1s, was associated with lower risk of all-cause death in some, but not all, cohorts (11). However, the association of these *GC* haplotypes with melanoma-specific death, and according to tumor Breslow thickness, has not been previously reported, to our knowledge. To confirm these associations, we expanded previous studies and used 2 population-based melanoma studies to investigate the associations of Gc1s, Gc1f, and Gc2 haplotypes with melanoma-specific and all-cause death, overall and according to tumor thickness.

Methods

Study population

We used data from 2 large, population-based melanoma cohorts: the international Genes, Environment and Melanoma (GEM) Study and the Western Australian Melanoma Health Study (WAMHS). Each study was approved by their respective institutional review board, and all participants provided written informed consent. Study details were published previously for GEM (12) and WAMHS (13). Briefly, GEM included 3579 incident primary cutaneous melanoma cases diagnosed between 1998 and 2003 in Australia, Canada, Italy, and the United States. WAMHS recruited 1643 incident primary invasive cutaneous melanoma cases diagnosed between 2006 and 2009 and identified through the Western Australian Cancer Registry.

Cohort data and follow-up

In the GEM Study, demographic and phenotypic data were collected using telephone interviews and self-administered questionnaires (12). Pathological data, including Breslow thickness and tumor site, were extracted from pathology reports. A centralized pathology review process was also conducted in GEM to obtain additional pathological data such as tumor-infiltrating lymphocytes (14,15). In WAMHS, demographic and phenotypic characteristics were obtained from questionnaires administered by telephone interviews, and pathological data, including Breslow thickness, was extracted from the Western Australia Cancer Registry (13). For recruited individuals with a higher-order primary melanoma (ie, with a prior primary melanoma), we used the pathological characteristics of the "index" melanoma that brought the individual into the study and marked the start of follow-up.

In both studies, follow-up time was accumulated from the date of diagnosis of the index primary melanoma until the date of death or until the end of follow-up (censorship). In GEM, cause of death information was obtained from the National Death Index for the US study centers and cancer registries and/or municipal records for non-US study centers. Patient follow-up for vital status was complete to the end of 2007 for US and Australian centers and to the end of 2008 for Canada and Italy. In WAMHS, cause and date of death data through 2017 were obtained from the Western Australian Death Registrations, via annual updates from the Western Australian Cancer Registry, for these analyses.

Genotyping

The Gc1s, Gc1f, and Gc2 haplotypes are determined by 2 single nucleotide polymorphisms (SNPs) in the *GC* gene: rs7041 (NG_012837.3: g.57904T>G NP_001191235.1: p.Asp432Glu) and rs4588 (NG_012837.3: g.57915C>A NP_001191235.1: p.Thr436Lys). We used *GC* genotyping data previously collected in GEM and WAMHS. In GEM, DNA was extracted from buccal swabs, and *GC* SNPs were genotyped using the MassArray iPLEX platform (Agena Bioscience, San Diego, CA, USA; previously known as Sequenom) with standard quality control procedures described previously (4). In GEM, *GC* rs2282679 was used as a proxy for rs4588 ($r^2 = 1.0$, CEU population [Utah residents with North/West European Ancestry] [16]), since rs4588 genotyping data were not available. Both rs7041 and rs4588 were available in the WAMHS data, and the SNPs rs2282679 and rs4588 were in perfect linkage disequilibrium ($r^2 = 1.0$) in this cohort, further supporting rs2282679 as an appropriate proxy for rs4588. In WAMHS, DNA was extracted from peripheral blood samples and genotyped using the Illumina OmniXpressExome-v1 chip (Illumina, San Diego CA) with standard quality control procedures described previously (13,17).

The combined rs7041 and rs4588 (or rs2282679 proxy) genotypes were used to infer the 3 common haplotypes (Gc1s, Gc1f, Gc2) and the 6 resultant haplotype combinations (or diplotypes) observed in appreciable frequencies: Gc1s-1, Gc1s-1f, Gc1s-2, Gc2-1f, Gc1f-1f, and Gc2-2. Given the rarity of the rs7041*G + rs4588*A allele combination known as the Gcx haplotype (haplotype frequency in GEM and WAMHS < 0.001), the Gc2-1 diplotype was assumed for individuals with heterozygous genotypes at both SNPs, consistent with previous studies (18).

Exclusions

Of the 5222 melanoma cases recruited in GEM and WAMHS, we excluded 283 GEM cases of situ melanoma, 42 GEM cases and 390 WAMHS cases with missing *GC* genotype data, 11 GEM cases who self-reported non-European ancestry (to avoid potential population-stratification bias), 1 GEM case with missing follow-up data, and 5 GEM cases with the rare Gcx haplotype, leaving 4490 participants for analysis. In WAMHS, 2 individuals who self-reported non-European ancestry were included because they were deemed to be of European ancestry based on prior genetic principal component analyses for genome-wide association study analyses (17).

Statistical methods

Study-specific and pooled hazard ratios (HRs) and 95% confidence intervals (CIs) for death according to *GC* haplotype were estimated using Cox-proportional hazards regression. The proportional hazards assumption was assessed using Schoenfeld residuals and by including a time-dependent variable in the Cox model. Our primary exposure was the presence vs absence of the Gc1f haplotype (dominant inheritance model), which was chosen a priori based on findings of the aforementioned BioGenoMEL study (11) and given the low frequency of the Gc1f-1f diplotype (ie, Gc1f homozygotes; <5% reported in White populations of

European-ancestry [19,20]). In secondary analyses, we estimated the association of each GC diplotype with melanoma-specific and all-cause death using the most common Gc1s-1 diplotype as the reference group.

The HRs were estimated in a minimally adjusted model that included age, sex, first- or higher-order primary melanoma at recruitment, and study center; and a fully adjusted model that also included tumor site, phenotypic index (combining hair color, eye color, and ability to tan), and log of Breslow thickness (log transformed to normalize the heavily right-skewed Breslow thickness variable). Covariates were chosen based on biological plausibility, causal structure, and the previous literature (11,21). Variable coding details are provided in the table footnotes.

To assess potential effect modification, we estimated HRs in pooled, fully adjusted models according to site, first vs higher-order primary, and Breslow thickness equal to or less than 2.0 mm ("lower-risk" stages) vs greater than 2.0 mm ("higher-risk" stages) consistent with a prior GEM study (14). To visually assess whether competing causes of death may influence the observed associations, adjusted cumulative incidence curves for melanoma-specific deaths were estimated by using the Fine-Gray subdistribution hazard model competing-risks regression (22). In exploratory analyses, to investigate whether the association of Gc1f with survival may be mediated by prognostic histologic characteristics, we estimated the association of Gc1f with Breslow thickness in both cohorts and with other prognostic histologic characteristics (eg, ulceration, mitoses, tumor-infiltrating lymphocytes) that were only available in GEM.

Several sensitivity analyses were performed. Potential bias due to population stratification was assessed through principal component analysis using a set of low-penetrant melanoma-risk variants, previously selected for investigation in a pooled GEM and WAMHS study (23). We also investigated whether adding a self-reported ancestry variable (UK/Ireland, other Northern European, Southern European, mixed European, other/unknown European Ancestry) to the fully adjusted model changed the study-specific or pooled HRs. In GEM, a small number of patients with a first primary melanoma developed a second primary melanoma during follow-up (n = 96), so we performed a sensitivity analysis by adding a time-dependent covariate to the fully adjusted model.

All statistical tests were 2-sided; a *P* value less than .05 was considered statistically significant. Analyses were performed in R version 3.6.3 (R Foundation, Vienna, Austria).

Results

Of the 4490 individuals in the pooled cohort, 688 individuals died (15%) and 323 individuals died from melanoma (7%). Median follow-up times were 7.6 years in GEM and 9.1 years in WAMHS. Selected characteristics of study participants according to Gc1f haplotype are presented in Table 1**;** 1278 individuals (28%) carried the Gc1f haplotype. The SNP information, including genomic location, number genotyped, and minor allele frequencies, are presented in Supplementary Table, available online)

The associations of Gc1f with melanoma-specific and all-cause death are presented in Table 2. The HRs for melanoma-specific and all-cause death were similar in both the GEM and WAMHS cohorts when analyzed separately. In the pooled cohort and fully adjusted model, those with the Gc1f haplotype had a statistically significantly 37% lower risk of melanoma-specific death compared with those without Gc1f (HR = 0.63, 95% CI = 0.47 to 0.83). The corresponding pooled, fully adjusted HR for all-cause death was 0.89 (95% CI = 0.75 to 1.07).

Associations of each GC diplotype (ie, haplotype combinations) with melanoma-specific death in fully adjusted models in the pooled cohort are presented in Supplementary Table 2 (available online). The HRs for melanoma-specific death associated with the Gc1s-1f and Gc2-1f diplotypes (i.e, Gc1f heterozygotes) were 0.55 (95% CI = 0.38 to 0.80) and 0.67 (95% CI = 0.42 to 1.06), respectively, relative to the most common diplotype Gc1s-1. The corresponding HR associated with Gc1f-1f (ie, Gc1f homozygotes) was 0.42 (95% CI = 0.15 to 1.13) relative to Gc1s-1. The Gc2-containing diplotypes were inversely associated with melanoma-specific death relative to Gc1s-1, but these associations were not statistically significant (pooled HRs [95% CI]: 0.88 [0.67 to 1.06] for Gc2-1 and 0.75 [0.47 to 1.17] for Gc2-2).

The associations of Gc1f with melanoma-specific death in the pooled cohort stratified by Breslow thickness, tumor site, and first or higher-order primary melanoma are presented in Table 3. The melanoma-specific death HR associated with the presence vs absence of Gc1f was 0.89 (95% CI = 0.63 to 1.27) among participants with tumors of 2.0 mm Breslow thickness or less ("low-risk" stages) and 0.40 (95% CI = 0.25 to 0.63) among participants with tumors greater than 2.0 mm Breslow thickness ("high-risk" stages) ($P_{interaction}$ = .003). The association of Gc1f with melanoma-specific death did not differ statistically signifcantly by tumor site. Separating first- and higher-order primary groups showed virtually identical HR estimates in both groups, although the lower numbers of cases in the higher-order melanoma group resulted in wider CIs and non–statistially significant HRs.

The cumulative incidence of melanoma-specific death, accounting for competing causes of death, associated with Gc1f and stratified by Breslow thickness are shown in Figure 1. Consistent with our Cox proportional-hazards models, those with Gc1f had a lower cumulative incidence of melanoma-specific death relative to those without Gc1f among all participants combined (Figure 1, A); however, when stratified by Breslow thickness, this association was only apparent among cases with a Breslow thickness greater than 2.0 mm ("higher-risk" stages). Among these cases with a Breslow thickness greater than 2.0 mm, the cumulative incidence of melanoma death within 5 years was an estimated 12% (95% CI = 7% to 16%) for those with Gc1f compared with 25% (95% CI = 21% to 29%) for those without Gc1f (Figure 1, C), controlling for all other covariates and accounting for competing causes of death.

In exploratory analyses, the presence vs absence of Gc1f was not statistically significantly associated with the log of Breslow thickness in GEM or WAMHS using multivariable linear regression models adjusted for age, sex, study center, and whether participants had a first- or higher-order primary tumor (Supplementary Table 3, available online). Also, in GEM, presence vs absence of Gc1f was not statistically significantly associated with other prognostic histologic variables—mitoses, ulceration, solar elastosis, or tumor infiltrating lymphocytes—in models adjusted for age, sex, study center, and whether participants had a first or higher-order primary (Supplementary Table 4, available online). In sensitivity analyses, adjusting for the top 3 principal components or adjusting for self-reported European ancestry did not materially affect the association of the presence vs absence of Gc1f with melanoma-specific death (HR change = 0-0.01, results not shown in tables). Also, including a time-dependent covariate for the 96 GEM patients who developed a second primary tumor during follow-up did not change the Gc1f HR for melanoma-specific death.

Table 1. Characteristics of 4490 individuals with invasive cutaneous melanoma according to Gc1f haplotype inheritance in the GEM and WAMHS cohort studies[a]

Variable	Gc1f haplotype	
	Absent (n = 3212)	Present (n = 1278)
Study, No. (%)		
GEM	2321 (72)	916 (72)
WAMHS	891 (28)	362 (28)
GC diplotype, No. (%)		
Gc1s-1	1444 (45)	—
Gc2-1[b]	1392 (43)	—
Gc2-2	376 (12)	—
Gc2-1f	—	380 (30)
Gc1s-1f	—	793 (62)
Gc1f-1f	—	105 (8)
Age, median (IQR), y	59 (47-70)	60 (48-70)
Sex, No. (%)		
Male	1805 (56)	721 (56)
Female	1407 (44)	557 (44)
Breslow thickness, median (IQR), mm[c]	0.7 (0.4-1.2)	0.7 (0.4-1.3)
Log of Breslow thickness, median (IQR), mm	−0.4 (−0.9 to 0.2)	−0.4 (−0.8 to 0.3)
Breslow thickness categories, No. (%)		
≤2.0 mm ("low risk")	2745 (86)	1080 (85)
>2.0 mm ("high risk")	392 (12)	160 (13)
Missing	75 (2)	38 (3)
Site, No. (%)		
Head and neck	558 (17)	215 (17)
Trunk	1352 (42)	520 (41)
Upper extremities	627 (20)	263 (20)
Lower extremities	672 (21)	279 (22)
Missing	3 (0)	1 (0)
Phenotypic index[d], No. (%)		
0	241 (8)	85 (7)
1	642 (20)	225 (18)
2	1219 (38)	483 (38)
3	796 (25)	346 (27)
4	192 (6)	84 (7)
Missing	122 (4)	55 (4)
Primary melanoma status, No. (%)		
First primary melanoma	2521 (78)	980 (77)
Higher-order primary melanoma	691 (22)	298 (23)

[a] Percentages may not sum to 100 due to rounding. GEM = Genes, Environment and Melanoma study; HR = hazard ratio; IQR = interquartile range; SNP = single nucleotide polymorphism; WAMHS = Western Australia Melanoma Health Study.

[b] For patients with heterozygous genotypes at both SNPs, the Gc2-1 combined genotype was assumed (ie, rs7041*G + rs4588*C [Gc1s] on one chromosome and rs7041*T + rs4588*A [Gc2] on the homologous chromosome) as opposed to the other possible combination (ie, rs7041*T + rs4588*C [Gc1f] on one chromosome and rs7041*G + rs4588*A [Gcx] on the homologous chromosome), given the extreme rarity of the Gcx haplotype, consistent with other studies (Abbas et al, 2008 [18]).

[c] Among participants without Gc1f, 75 (2%) had missing Breslow thickness; among those with Gc1f, 38 (3%) had missing Breslow thickness.

[d] Factor variable created by combining the following eye colo:r [black/brown (0), blue/green/other (1)], hair color [black/dark brown (0), light brown/blonde (1), red (2)], and tannability [deeply/moderate (0), little/none (1)]. A higher index indicates greater pigmentary melanoma risk factors.

Discussion

This study it the first, to our knowledge, to report *GC* haplotype associations with melanoma-specific death. In a previous meta-analysis including 2565 melanoma cases in Europe and the United States (BioGenoMEL consortium), Gc1s and Gc2, relative to Gc1f, were associated with higher overall (all-cause) death—Gc1s vs Gc1f HR = 1.17 (95% CI = 0.95 to 1.43) and Gc1s vs Gc1f HR = 1.28 (95% CI = 0.88 to 1.86) (11)—but these associations did not attain statistical significance. Melanoma-specific death was not available in all BioGenoMEL cohorts and was not reported for each haplotype. In our study, those with the Gc1f haplotype had a statistically significantly lower risk of melanoma-specific death but not overall death compared with those without Gc1f, although the pooled HR for overall death suggested consistency with findings from BioGenomel. Our findings suggest that inheritance of the Gc1f haplotype may be more strongly inversely associated with risk of death attributable to melanoma, rather than other causes, among melanoma patients. Moreover, this survival advantage associated with Gc1f may be restricted to higher-risk cases with tumors thicker than 2.0 mm, corresponding to tumor (T) stages T3/T4 in the American Joint Commission on Cancer (AJCC) 8th edition.

Multiple vitamin D–related biomarkers—including $25(OH)D_3$ concentrations (2), VDR expression (6), and expression of the vitamin D–activating CYP27B1 enzyme (24)—have been associated with melanoma progression and prognosis. Higher circulating levels of $25(OH)D_3$ were inversely associated with Breslow thickness and melanoma-specific death (independently of Breslow thickness) in a prior prospective cohort study (2). Intriguingly, Gc1f is associated with higher circulating $25(OH)D_3$ levels relative to the other haplotypes, particularly relative to Gc2, which may be mediated by higher DBP concentrations (25,26). However, we suspect that this *GC* haplotype is unlikely to account for sufficient variability in $25(OH)D_3$ (eg, $r^2 < 0.1$ [27]) to account for its association with melanoma-specific death. Hibler et al (28) found that $1,25(OH)_2D$ uptake in colon cancer cells significantly differed by *GC* haplotype, and that the Gc1f-1 and Gc1f-2 diplotypes produced the greatest VDR pathway activation by $1,25(OH)_2D$. However, the effects of *GC* haplotypes on VDR activation in melanoma and on other important vitamin D derivatives [eg, $20(OH)D_3$ and $1,20(OH)_2D_3$ metabolized by CYP11A1 and with demonstrated antineoplastic effects in melanocytes] are unclear (29,30).

Beyond its role in vitamin D transport, DBP can be converted into the potent macrophage-activating factor known as GcMAF through posttranslational glycosylation modifications (31). In laboratory studies, GcMAF-activated tumoricidal macrophages and inhibited angiogenesis and cell proliferation in breast and prostate cancer cell lines (9,32). Additionally, Gc1f was associated with increased GcMAF precursor activity, relative to Gc1s and Gc2, which may be due to differences in the glycan-binding to domain III of DBP affected by the amino acid changes at positions 432 and 436 (33). However, the role of GcMAF and possible haplotype-specific GcMAF activities on melanoma progression are unknown.

Strengths of this study included the prospective study design, long follow-up periods, investigation of melanoma-specific and all-cause death, and use of data from 2 large independently conducted studies with population-based recruitment in the United States, Canada, Italy, and Australia.

This study has several limitations. Complete AJCC tumor staging data was only available in GEM; however, Breslow thickness, the most important prognostic factor in AJCC staging, was controlled for in both cohorts. Within GEM, further adjusting for AJCC stage in the fully adjusted model did not materially affect the Gc1f HR estimates. We did not measure circulating 25(OH)D concentrations, so the degree to which haplotype-associated differences in 25(OH)D may mediate the association of Gc1f with melanoma-specific death is unknown. Nor did we measure other hydroxyvitamin D derivates involved in alternative vitamin D

Table 2. Study-specific and pooled hazard ratios for melanoma-specific and all-cause death according to Gc1f haplotype inheritance in the GEM and WAMHS cohorts (n = 4203)[a]

	No. of deaths/total No. (%)		Present vs absent Gc1f haplotype	
Outcome variable and study	Gc1f absent	Gc1f present	HR (95% CI)	P
Melanoma-specific death				
Minimally adjusted[b]				
GEM	173/2196 (7.8%)	48/860 (5.6%)	0.71 (0.51 to 0.98)	.03
WAMHS	57/821 (6.9%)	17/326 (5.2%)	0.74 (0.43 to 1.28)	.28
Pooled	230/3017 (7.6%)	65/1186 (5.4%)	0.71 (0.54 to 0.94)	.02
Fully adjusted[c]				
GEM	173/2196 (7.8%)	48/860 (5.6%)	0.61 (0.44 to 0.85)	.003
WAMHS	57/821 (6.9%)	17/326 (5.2%)	0.64 (0.37 to 1.12)	.12
Pooled	230/3017 (7.6%)	65/1186 (5.4%)	0.63 (0.47 to 0.83)	.001
All-cause death				
Minimally adjusted[b]				
GEM	346/2196 (15.8%)	125/860 (14.5%)	0.95 (0.77 to 1.17)	.62
WAMHS	109/821 (13.3%)	48/326 (14.7%)	1.04 (0.74 to 1.47)	.81
Pooled	455/3017 (15.1%)	173/1186 (14.6%)	0.97 (0.81 to 1.16)	.75
Fully adjusted[c]				
GEM	346/2196 (15.8%)	125/860 (14.5%)	0.86 (0.70 to 1.06)	.16
WAMHS	109/821 (13.3%)	48/326 (14.7%)	0.98 (0.70 to 1.39)	.93
Pooled	455/3017 (15.1%)	173/1186 (14.6%)	0.89 (0.75 to 1.07)	.22

[a] Limited to 4203 participants with no missing data for any variables in the fully adjusted model. CI = confidence interval; GEM = Genes, Environment and Melanoma study; HR = hazard ratio; WAMHS = Western Australia Melanoma Health Study.
[b] Adjusted for age (continuous), sex, study center, and whether a first- or higher-order primary melanoma at recruitment.
[c] Adjusted for age (continuous), sex, study center, whether a first- or higher-order primary melanoma, site (head/neck, trunk, arms, legs), log of Breslow thickness (continuous), and phenotypic index (categories 0 to 4).

Table 3. Pooled hazard ratios for melanoma-specific death associated with Gc1f haplotype inheritance stratified by potential effect-modifiers in the pooled GEM and WAMHS cohorts (n = 4203)[a]

	No. of deaths/no. total (%)		Present vs absent Gc1f haplotype		
Strata or subgroup	*Gc1f* absent	*Gc1f* present	HR (95% CI)	P	$P_{interaction}$[e]
Site[b]					
Head/neck	73/512 (14.3%)	18/198 (9.1%)	0.47 (0.27 to 0.81)	.01	
Trunk	97/1286 (7.5%)	25/488 (5.1%)	0.61 (0.38 to 0.96)	.03	
Extremities	60/1219 (4.9%)	22/500 (4.4%)	0.89 (0.54 to 1.46)	.52	.43
Breslow thickness, mm[c]					
≤2.0	116/2644 (4.4%)	42/1035 (4.1%)	0.89 (0.63 to 1.27)	.19	
>2.0	114/373 (30.6%)	23/151 (15.2%)	0.40 (0.25 to 0.63)	<.001	.003
Primary status at recruitment[d]					
First primary	164/2372 (6.9%)	41/914 (4.5%)	0.63 (0.44 to 0.89)	.008	
Second or higher-order primary	66/645 (10.2%)	24/272 (8.8%)	0.65 (0.40 to 1.05)	.08	.90

[a] Limited to 4203 participants with available phenotypic index data (combining hair color, eye color and tannability). CI = confidence interval; GEM = Genes, Environment and Melanoma study; HR = hazard ratio; No. = number; WAMHS = Western Australia Melanoma Health Study.
[b] HRs by site estimated in Cox proportional hazards models adjusted for age (continuous), sex, study center, whether a first or higher-order primary melanoma, log of Breslow thickness (continuous), and phenotypic index (categories 0 to 4).
[c] HRs by Breslow category estimated in Cox proportional hazards models adjusted for age (continuous), sex, study center, whether a first or higher-order primary melanoma, log of Breslow thickness, site (head/neck, trunk, arms, legs), and phenotypic index (categories 0 to 4).
[d] HRs by primary status estimated in Cox proportional hazards models adjusted for age (continuous), sex, study center, log of Breslow thickness, site (head/neck, trunk, arms, legs), and phenotypic index (categories 0 to 4).
[e] $P_{interaction}$ calculated using a log-likelihood test comparing the multivariable-adjusted model with and without the interaction term.

activation pathways, such as those metabolized by CYP11A1, as these measurements were beyond the scope of this study (29,34). Potential population stratification bias was considered because Gc1f is strongly associated with ancestry and is more common in Black populations of African ancestry than White populations of European ancestry (19). However, analyses were restricted to individuals of European ancestry, and further adjustment for self-reported ancestry and top principal components did not materially affect our results. Furthermore, since melanomas arising in Black individuals are associated with more advanced stages and poorer prognosis than those arising in White individuals (35), potential uncontrolled confounding by race/ethnicity may be expected to bias the HR estimates for Gc1f toward the null. As this was a hypothesis-driven study with a priori SNPs, we did not adjust for multiple comparisons; thus, our results may need to be interpreted with caution. Last, there may be exposure misclassification due to genotyping error or incorrect inference of the *GC* haplotype for those with heterozygous genotypes at both SNPs; however, we would expect this misclassification to be small, nondifferential with respect to the outcome, and likely to weaken the estimated HRs toward the null.

In summary, our findings suggest that patients with invasive cutaneous melanoma who inherit the Gc1f haplotype, determined by 2 missense variants in the DBP-encoding gene *GC*, may be less likely to die as a result of melanoma than melanoma patients without Gc1f. This association may be restricted to patients with thicker tumors who are at a higher overall risk of death. Future studies are needed to investigate the role of DBP in

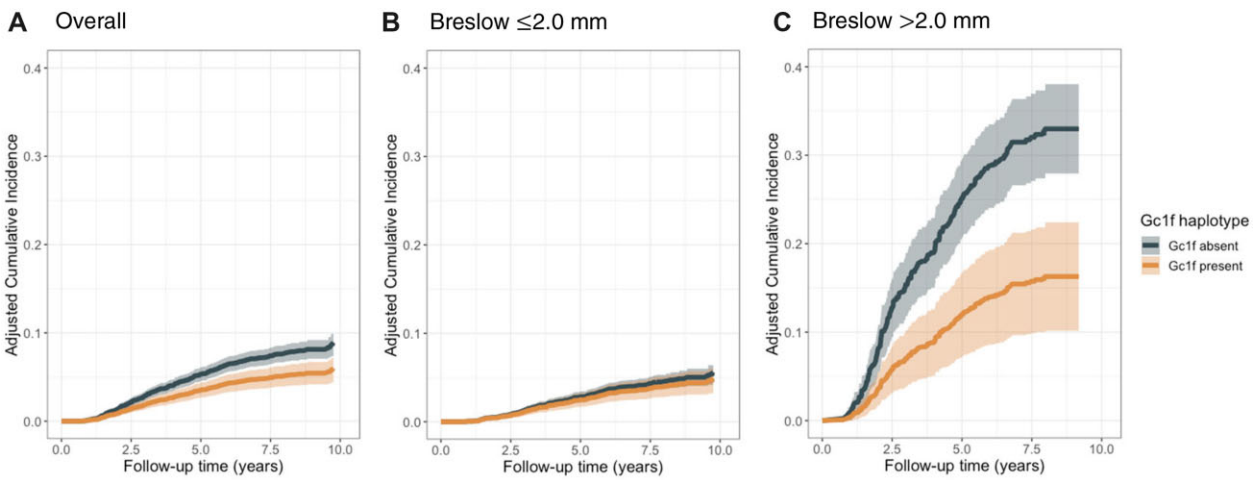

Figure 1. Adjusted cumulative incidence estimates and 95% confidence intervals of melanoma-specific death, accounting for competing causes of death, among (**A**) all participants (n = 4203), (**B**) participants with equal to or less than 2.0-mm–thick tumors (n = 3679), and (**C**) participants with greater than 2.0-mm–thick tumors (n = 524). Models adjusted for age, sex, whether a first or higher-order primary melanoma, study center, log of Breslow thickness, and phenotypic index.

melanoma progression and the clinical utility of this GC haplotype as a potential new prognostic factor for melanoma.

Data availability

Data may be made available upon request to the Corresponding Author and pending review by the GEM and WAMHS Steering Committees.

Author contributions

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Conflicts of interest

None declared.

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References

1. Feldman D, Krishnan AV, Swami S, et al. The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer*. 2014;14(5):342-357.
2. Newton-Bishop JA, Beswick S, Randerson-Moor J, et al. Serum 25-hydroxyvitamin D3 levels are associated with Breslow thickness at presentation and survival from melanoma. *J Clin Oncol*. 2009;27(32):5439-5444.
3. Caini S, Boniol M, Tosti G, et al. Vitamin D and melanoma and non-melanoma skin cancer risk and prognosis: a comprehensive review and meta-analysis. *Eur J Cancer*. 2014;50(15):2649-2658.
4. Orlow I, Roy P, Reiner AS, et al.; GEM Study Group. Vitamin D receptor polymorphisms in patients with cutaneous melanoma. *Int J Cancer*. 2012;130(2):405-418.
5. Hutchinson PE, Osborne JE, Lear JT, et al. Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma. *Clin Cancer Res*. 2000;6(2):498-504.
6. Brożyna AA, Jóźwicki W, Slominski AT. Decreased VDR expression in cutaneous melanomas as marker of tumor progression: new data and analyses. *Anticancer Res*. 2014;34(6):2735-2743.
7. Bikle DD, Gee E, Halloran B, et al. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. *J Clin Endocrinol Metab*. 1986;63(4):954-959.
8. Kisker O, Onizuka S, Becker CM, et al. Vitamin D binding protein-macrophage activating factor (DBP-maf) inhibits angiogenesis and tumor growth in mice. *Neoplasia*. 2003;5(1):32-40.
9. Pacini S, Punzi T, Morucci G, et al. Effects of vitamin D-binding protein-derived macrophage-activating factor on human breast cancer cells. *Anticancer Res*. 2012;32(1):45-52.
10. Malik S, Fu L, Juras DJ, et al. Common variants of the vitamin D binding protein gene and adverse health outcomes. *Crit Rev Clin Lab Sci*. 2013;50(1):1-22.
11. Davies JR, Field S, Randerson-Moor J, et al. An inherited variant in the gene coding for vitamin D-binding protein and survival from cutaneous melanoma: a BioGenoMEL study. *Pigment Cell Melanoma Res*. 2014;27(2):234-243.
12. Begg CB, Hummer AJ, Mujumdar U, et al.; GEM Study Group. A design for cancer case–control studies using only incident cases: experience with the GEM study of melanoma. *Int J Epidemiol*. 2006;35(3):756-764.
13. Ward SV, Cadby G, Lee A, et al. The Western Australian Melanoma Health Study: study design and participant characteristics. *Cancer Epidemiol*. 2011;35(5):423-431.
14. Thomas NE, Edmiston SN, Alexander A, et al.; GEM Study Group. Association between NRAS and BRAF mutational status and melanoma-specific survival among patients with higher-risk primary melanoma. *JAMA Oncol*. 2015;1(3):359-368.
15. Thomas NE, Busam KJ, From L, et al. Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based genes, environment and melanoma study. *J Clin Oncol*. 2013;31(33):4252-4259.
16. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*. 2015;31(21):3555-3557.
17. Law MH, Bishop DT, Lee JE, et al.; ATHENS Melanoma Study Group. Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. *Nat Genet*. 2015;47(9):987-995.
18. Abbas S, Linseisen J, Slanger T, et al. The Gc2 allele of the vitamin D binding protein is associated with a decreased postmenopausal breast cancer risk, independent of the vitamin D status. *Cancer Epidemiol Biomarkers Prev*. 2008;17(6):1339-1343.
19. Kamboh M, Ferrell R. Ethnic variation in vitamin D-binding protein (GC): a review of isoelectric focusing studies in human populations. *Hum Genet*. 1986;72(4):281-293.
20. Gibbs DC, Fedirko V, Um C, et al. Associations of circulating 25-hydroxyvitamin D3 concentrations with incident, sporadic colorectal adenoma risk according to common vitamin D binding protein isoforms. *Am J Epidemiol*. 2018;187(9):1923-1930.
21. Ward SV, Gibbs DC, Orlow I, et al.; GEM Study Group. Association of IRF4 single-nucleotide polymorphism rs12203592 with melanoma-specific survival. *Br J Dermatol*. 2020;183(1):163-165.
22. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc*. 1999;94(446):496-509.
23. Gibbs DC, Ward SV, Orlow I, et al.; GEM Study Group. Functional melanoma-risk variant IRF4 rs12203592 associated with Breslow thickness: a pooled international study of primary melanomas. *Br J Dermatol* 2017;177(5):e180-e182.
24. Brożyna AA, Jóźwicki W, Janjetovic Z, et al. Expression of the vitamin D-activating enzyme 1α-hydroxylase (CYP27B1) decreases during melanoma progression. *Hum Pathol*. 2013;44(3):374-387.
25. Lauridsen AL, Vestergaard P, Hermann A, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1, 25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. *Calcif Tissue Int*. 2005;77(1):15-22.
26. Lauridsen AL, Vestergaard P, Nexo E. Mean serum concentration of vitamin D-binding protein (Gc globulin) is related to the Gc phenotype in women. *Clin Chem*. 2001;47(4):753-756.

27. Powe CE, Karumanchi SA, Thadhani R. Vitamin D-binding protein and vitamin D in blacks and whites. *N Engl J Med*. 2014; 370(9):880-881.
28. Hibler EA, Jacobs ET, Stone AD, et al. Associations between vitamin D–binding protein isotypes, circulating 25 (OH) D levels, and vitamin D metabolite uptake in colon cancer cells. *Cancer Prev Res (Phila)*. 2014;7(4):426-434.
29. Slominski AT, Brożyna AA, Zmijewski MA, et al. Vitamin D signaling and melanoma: role of vitamin D and its receptors in melanoma progression and management. *Lab Invest*. 2017;97(6):706-724.
30. Slominski AT, Kim TK, Shehabi HZ, et al. In vivo evidence for a novel pathway of vitamin D_3 metabolism initiated by P450scc and modified by CYP27B1. *FASEB J*. 2012;26(9):3901-3915.
31. Yamamoto N, Homma S. Vitamin D3 binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes. *Proc Natl Acad Sci USA*. 1991;88(19):8539-8543.
32. Gregory KJ, Zhao B, Bielenberg DR, et al. Vitamin D binding protein-macrophage activating factor directly inhibits proliferation, migration, and uPAR expression of prostate cancer cells. *PLoS One*. 2010;5(10):e13428.
33. Nagasawa H, Sasaki H, Uto Y, et al. Association of the macrophage activating factor (MAF) precursor activity with polymorphism in vitamin D-binding protein. *Anticancer Res*. 2004;24(5c): 3361-3366.
34. Slominski AT, Chaiprasongsuk A, Janjetovic Z, et al. Photoprotective properties of vitamin D and lumisterol hydroxyderivatives. *Cell Biochem Biophys*. 2020;78(2):165-180.
35. Rouhani P, Hu S, Kirsner RS. Melanoma in Hispanic and black Americans. *Cancer Control*. 2008;15(3):248-253.