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 three common haplotypes Gc1s, Gc1f, and Gc2. Gc1f may be associated with decreased all-cause death among melanoma patients, based on results of a prior study, but its association 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 4,490 individuals with incident, invasive primary melanoma in two population-based studies using multivariable Cox-proportional hazards regression.

Results: In the pooled analysis of both datasets, those with the Gc1f haplotype had a 37% lower risk of melanoma-specific death compared to those without Gc1f (hazard ratio [HR] = 0.63, 95% confidence interval [CI] = 0.47 to 0.83, $P = 0.001$) adjusting 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 with the Gc1f haplotype compared to those without Gc1f were 0.89 (95% CI = 0.63 to 1.27) among participants with tumors ≤2.0 mm and 0.40 (95% CI = 0.25 to 0.63) among participants with tumors >2.0 mm ($P_{interaction} = 0.003$).

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—compared to those without Gc1f.

Introduction

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 (25[OH]D_3]) concentrations—the primary circulating form of vitamin D and 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.

Nearly 90% of circulating 25(OH)D_3 is bound to the DBP, which can affect vitamin D half-life and bioavailability to target tissues (7). DBP can also be converted into a macrophage-activating factor (GcMAF) 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 inherited *GC* haplotypes Gc1s, Gc1f, and Gc2 (also known as DBP1s, DBP1f, DBP2) encoded by two *GC* missense variants altering the amino acid sequence at position 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 epidemiologic study of nine 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 reported to

our knowledge. To address this, we expanded previous studies and used two population-based melanoma studies to investigate the association 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 two 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 recruited 3,579 incident primary cutaneous melanoma cases diagnosed between 1998 and 2003 in Australia, Canada, Italy, and the United States (US). WAMHS recruited 1,643 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 GEM, demographic and phenotypic data were collected using telephone interviews and self-administered questionnaires (12). Pathologic data, including Breslow thickness and tumor site, were exactred from pathology reports. A centralized pathology review process was also conducted in GEM to obtain additional pathologic data such as tumor infiltrating lymphocytes (14, 15). In WAMHS, demographic and phenotypic characteristics were obtained by questionnaires administered by telephone interviews, and pathological data, including

Breslow thickness, was extracted from the Western Australia Cancer Registry (13). For individuals recruited with a higher-order primary melanoma (i.e., with a prior primary melanoma), we used the pathologic 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 were obtained from the Western Australian Death Registrations, via annual updates from the Western Australian Cancer Registry, through 2017 for these analyses.

Genotyping

The Gc1s, Gc1f, and Gc2 haplotypes are determined by two single nucleotide polymorphism (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 was 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-1s, 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 Gc2-1s diplotype was assumed for individuals with heterozygous genotypes at both SNPs, consistent with previous studies (18).

Exclusions

Of the 5,222 melanoma cases recruited in GEM and WAMHS, we excluded 283 GEM cases with *in 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 4,490 participants for analysis. In WAMHS, 2 individuals who self-reported reported non-European ancestry were included as they were deemed to be of European ancestry based on prior genetic principal component analyses (PCA) for genome-wide association study analyses (19).

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 *versus* (*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 (i.e., Gc1f homozygotes; <5% reported in white populations of European-ancestry (20, 21)). In secondary analyses, we estimated the association of each *GC* diplotype with melanoma-specific and all-cause death using the most common Gc1s-1s 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 biologic plausibility, causal structure, and the previous literature (11, 22). 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 of ≤2.0 mm ("lower-risk" stages) *vs.* >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 death were estimated using Fine and Gray's competing-risks regression (23). In exploratory analyses, to investigate whether the

association of Gc1f with survival may be mediated by prognostic histologic characterstics, we estimated the the association of Gc1f with Breslow thickness in both cohorts and with other prognostic histologic characterstics (e.g., 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 (24). 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 first primary melanoma cases developed a second primary 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 two-sided; a *P*-value < 0.05 was considered statistically significant. Analyses were performed in R version 3.6.3 (R Foundation, Vienna, Austria).

Results

Of the 4,490 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;** 1,278 individuals (28%) carried the Gc1f haplotype. The SNP information including genomic location, number genotyped, and minor allele frequencies are presented in **Supplementary Table 1.**

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 to 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 (i.e., haplotype combinations) with melanoma-specific death in fully-adjusted models in the pooled cohort are presented in **Supplementary Table 2**. 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-1s . The corresponding HR associated with Gc1f-1f (i.e., Gc1f homozygotes) was 0.42 (95% CI = 0.15 to 1.13) relative to Gc1s-1s. The Gc2-containing diplotypes were inversely associated with melanoma-specific death relative to Gc1s-1s, but these associations were not statistically significant (pooled HRs [95% CI]: 0.88 [0.67 to 1.06] for Gc2-1s 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 ≤2.0 mm Breslow thickness ("low-risk" stages) and 0.40 (95% CI = 0.25 to 0.63) among participants with >2.0 mm Breslow thickness ("high-risk" stages) ($P_{interaction}$ = 0.003). The association of Gc1f with melanoma-specific death did not statistically signifcantly differ 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 confidence intervals and a non-statistially significant HR.

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 1A), however, when stratified by Breslow thickness, this association was only apparent among cases with a Breslow thickness >2.0 mm ("higher-risk" stages). Among these cases with a Breslow thickness >2.0 mm, the cumulative incidence of melanoma death within five years was an estimated 12% (95% CI = 7% to 16%) for those with Gc1f compared to 25% (95% CI = 21% to 29%) for those without Gc1f (Figure 1C), 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 (**Supplementary Table 3**). 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)**. In sensitivity analyses, adjusting for the top three principal components or adjusting for self-reported European ancestry did not materially affect the association of the presence *vs.* absence Gc1f with melanoma-specific death (HR change by 0-0.01, results not shown in tables). Also, including a time-dependent covariate for the 96 GEM

cases who developed a second primary during follow-up did not change the Gc1f HR for melanoma-specific death.

Discussion

This is the first study, to our knowledge, to report *GC* haplotype associations with melanoma-specific death. In a previous meta-analysis including 2,565 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 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 to 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 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 (25)—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 (26, 27). However, we suspect that this *GC* haplotype is unlikely to account for sufficient variability in $25(OH)D_3$ (e.g., $r^2 < 0.1$ (28)) for it to explain its association with melanoma-specific death. Hibler *et al.* (29) found that $1,25(OH)_2D$ uptake in colon cancer cells signficantly differed by *GC* haplotype, and that the Gc1f-1s 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 anti-neoplastic effects in melanocytes) are unclear (30, 31). .

Beyond its role in vitamin D transport, DBP can be converted into the potent macrophage activating factor known as GcMAF through post-translational glycosylation modifications (32). In laboratory studies, GcMAF activated tumoricidal macrophages and inhibited angiogenesis and cell proliferation in breast and prostate cancer cell lines (9, 33). Additionally, Gc1f was associated with increased GcMAF precursor activity, relative to Gc1s and Gc2, which may be due to differences in glycan-binding to domain III of DBP affected by the amino acid changes at position 432 and 436 (34). 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 two, 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, like those metabolized by CYP11A1, involved in alternative vitamin D activation pathways as it was beyond the scope of this study (30, 35). Potential population-stratification bias was considered since Gc1f is strongly associated with ancestry and is more common in Black populations of African ancestry than White populations of European ancestry (20). However, analyses were restricted to individuals of European ancestry and further adjusting for self-reported ancestry and top principal components did not materially affect our results. Furthermore, since melanomas arising in Black individuals, compared to White individuals, are associated with more advanced stages and poorer prognosis (36), one may expect potential uncontrolled confounding by race/ethnicity to bias the HR estimates for Gc1f towards 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, non-differential with respect to the outcome, and to weaken the estimated HRs towards the null.

In summary, our findings suggest that patients with invasive cutaneous melanoma who inherit the Gc1f haplotype, determined by two missense variants in the DBP-encoding gene *GC*, may be less likely to die as a result of melanoma compared to those 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 melanoma progression and the clinical utility of this *GC* haplotype as a potential new prognostic factor for melanoma.

Funding

This work was supported by the National Cancer Institute (R01CA233524 to N.E.T., M.B, C.B., and H.A.; P01CA206980 to N.E.T. and M.B; R01CA112243 to N.E.T.; U01CA83180 and R01CA112524 to M.B.; R01CA098438 to C.B.; R03CA125829 and R03CA173806 to I.O.; P30CA016086 to the University of North Carolina; and P30CA008748 to Memorial Sloan Kettering). This work was also supported by the National Institute of Environmental Health Sciences under award number P30ES010126, the University of North Carolina Center for Environmental Health and Susceptibility. A.E.C. was supported by a NHMCR Career Development Fellowship. S.V.W. was supported by an NHMCR Early Career Fellowship.

Notes

Conflicts of Interest Disclosures: None declared.

Acknowledgements: We are indebted to the GEM Study Group: Coordinating Center, Memorial Sloan Kettering Cancer Center, New York, NY (USA): Marianne Berwick, M.P.H., Ph.D. (PI, University of New Mexico, Albuquerque, NM), Colin Begg, Ph.D. (co-PI), Irene Orlow, D.Sc., M.S. (co-Investigator), Klaus J. Busam, M.D. (Dermatopathologist), Isidora Autuori (Research Assistant), Audrey Mauguen, Ph.D. (Biostatistician). Germline DNA handling, and genotyping design and testing for this study specifically were completed by Pampa Roy, Ph.D. (Senior Laboratory Technician), Sarah Yoo, M.A. (Senior Laboratory Technician), Ajay Sharma, M.S. (Senior Laboratory Technician), and Jaipreet Rayar, M.S. (Senior Laboratory Technician). University of New Mexico, Albuquerque, NM: Marianne Berwick, M.P.H., Ph.D. (PI), Li Luo, Ph.D. (Biostatistician), Tawny W. Boyce, M.P.H. (Data Manager). Study Centers: The University of Sydney and The Cancer Council New South Wales, Sydney, Australia: Anne E. Cust, Ph.D. (PI), Bruce K. Armstrong, M.D., Ph.D. (former PI), Anne Kricker, Ph.D., (former co-PI); Menzies Institute for Medical Research University of Tasmania, Hobart, Australia: Alison Venn, Ph.D. (current PI), Terence Dwyer, M.D. (PI, currently at University of Oxford, United Kingdom), Paul Tucker (Dermatopathologist); BC Cancer Research Centre, Vancouver, Canada: Richard P. Gallagher, M.A. (PI); Cancer Care Ontario, Toronto, Canada: Loraine D. Marrett, Ph.D. (PI); CPO, Center for Cancer Prevention, Torino, Italy: Roberto Zanetti, M.D. (PI), Stefano Rosso, M.D., M.Sc. (co-PI), Lidia Sacchetto, Ph.D. (Biostatistician); University of California, Irvine, CA: Hoda Anton-Culver, Ph.D. (PI); University of Michigan, Ann Arbor, MI:

Stephen B. Gruber, M.D., M.P.H., Ph.D. (PI, currently at City of Hope National Medical Center, Duarte, CA), Shu-Chen Huang, M.S., M.B.A. (co-Investigator, joint at USC-University of Michigan); University of North Carolina, Chapel Hill, NC: Nancy E. Thomas, M.D., Ph.D. (PI), Kathleen Conway, Ph.D. (co-Investigator), David W. Ollila, M.D. (co-Investigator), Paul B. Googe, M.D. (Dermatopathologist), Sharon N. Edmiston, B.A. (Research Analyst), Honglin Hao (Laboratory Specialist), Eloise Parrish, M.S.P.H. (Laboratory Specialist), David C. Gibbs, M.D., Ph.D. (Resident Physician at Emory University, Atlanta, GA); University of Pennsylvania, Philadelphia, PA: Timothy R. Rebbeck, Ph.D. (former PI), Peter A. Kanetsky, M.P.H., Ph.D. (PI, currently at H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL); UV data consultants: Julia Lee Taylor, Ph.D. and Sasha Madronich, Ph.D., National Centre for Atmospheric Research, Boulder, CO. The WAMHS gratefully acknowledges all particpiants for their time and contributions, as well as the Western Australian DNA Bank at the University of Western Australia, the Western Australian Cancer Registry and the WAMHS study team.

The study sponsors had no role in the design and conduct of the study; the analysis and interpretation of the data; the preparation, review or approval of the manuscript; nor the decision to submit the manuscript for publication.

Data Availability Statement

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

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

	Gc1f haplotype	
Variable	Absent (n = 3212)	Present (n = 1278)
Study, n (%)		
GEM	2321 (72)	916 (72)
WAMHS	891 (28)	362 (28)
GC diplotype, n (%)		
Gc1s-1s	1444 (45)	--
Gc2-1s[b]	1392 (43)	--
Gc2-2	376 (12)	--
Gc2-1f	--	380 (30)
Gc1s-1f	--	793 (62)
Gc1f-1f	--	105 (8)
Age		
Median (IQR)	59 (47, 70)	60 (48, 70)
Sex, n (%)		
Male	1805 (56)	721 (56)
Female	1407 (44)	557 (44)
Breslow thickness[c]		
Median (IQR)	0.7 (0.4, 1.2)	0.7 (0.4, 1.3)
Log of Breslow thickness[c]		
Median (IQR)	-0.4 (-0.9, 0.2)	-0.4 (-0.8, 0.3)
Breslow thickness categories, n (%)		
≤2.0 mm (“low risk”)	2745 (86)	1080 (85)
>2.0 mm (“high risk”)	392 (12)	160 (13)
Missing	75 (2)	38 (3)
Site, n (%)		
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], n (%)		
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, n (%)		
First primary melanoma	2521 (78)	980 (77)

Higher-order primary melanoma	691 (22)	298 (23)

Abbreviations: GEM, Genes, Environment and Melanoma study; HR, hazard ratio; IQR interquartile range; n, number; WAMHS, Western Australia Melanoma Health Study

[a]Data presented as number (%) unless otherwise noted. Percentages may not sum to 100 due to rounding.

[b]For patients with heterozygous genotypes at both SNPs, the Gc2-1s combined genotype was assumed (i.e., 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 (i.e., 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).

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

[d]Factor variable created by combining: eye color [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.

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. deaths / no. total (%)		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)	0.03
WAMHS	57/821 (6.9%)	17/326 (5.2%)	0.74 (0.43 to 1.28)	0.28
Pooled	230/3017 (7.6%)	65/1186 (5.4%)	0.71 (0.54 to 0.94)	0.02
Fully-adjusted[c]				
GEM	173/2196 (7.8%)	48/860 (5.6%)	0.61 (0.44 to 0.85)	0.003
WAMHS	57/821 (6.9%)	17/326 (5.2%)	0.64 (0.37 to 1.12)	0.12
Pooled	230/3017 (7.6%)	65/1186 (5.4%)	0.63 (0.47 to 0.83)	0.001
All-cause death				
Minimally-adjusted[b]				
GEM	346/2196 (15.8%)	125/860 (14.5%)	0.95 (0.77 to 1.17)	0.62
WAMHS	109/821 (13.3%)	48/326 (14.7%)	1.04 (0.74 to 1.47)	0.81
Pooled	455/3017 (15.1%)	173/1186 (14.6%)	0.97 (0.81 to 1.16)	0.75
Fully-adjusted[c]				
GEM	346/2196 (15.8%)	125/860 (14.5%)	0.86 (0.70 to 1.06)	0.16
WAMHS	109/821 (13.3%)	48/326 (14.7%)	0.98 (0.70 to 1.39)	0.93
Pooled	455/3017 (15.1%)	173/1186 (14.6%)	0.89 (0.75 to 1.07)	0.22

Abbreviations: CI, confidence interval; GEM, Genes, Environment and Melanoma study; HR, hazard ratio; No., number; WAMHS, Western Australia Melanoma Health Study; vs., versus

[a]Limited to 4203 participants with no missing data for any variables in the fully-adjusted model.

[b]Adjusted for age (continuous), sex, study centre, and whether a first or higher-order primary melanoma at recruitment.

[c]Adjusted for age (continuous), sex, study centre, whether a first or higher-order primary melanoma, site (head/neck, trunk, arms, legs), log of Breslow thickness (continuous), and phenotypic index (categories 0-4).

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. 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)	0.01	
Trunk	97/1286 (7.5%)	25/488 (5.1%)	0.61 (0.38 to 0.96)	0.03	
Extremities	60/1219 (4.9%)	22/500 (4.4%)	0.89 (0.54 to 1.46)	0.52	0.43
Breslow thickness, mm[c]					
≤2.0	116/2644 (4.4%)	42/1035 (4.1%)	0.89 (0.63 to 1.27)	0.19	
>2.0	114/373 (30.6%)	23/151 (15.2%)	0.40 (0.25 to 0.63)	<0.001	0.003
Primary status at recruitment[d]					
First primary	164/2372 (6.9%)	41/914 (4.5%)	0.63 (0.44 to 0.89)	0.008	
Second or higher-order primary	66/645 (10.2%)	24/272 (8.8%)	0.65 (0.40 to 1.05)	0.08	0.90

Abbreviations: CI, confidence interval; GEM, Genes, Environment and Melanoma study; HR, hazard ratio; No., number; WAMHS, Western Australia Melanoma Health Study; vs., versus

[a]Limited to 4203 participants with available phenotypic index data (combining hair color, eye color and tannability).

[b]HRs by site estimated in Cox proportional hazards models adjusted for age (continuous), sex, study centre, whether a first or higher-order primary melanoma, log of Breslow thickness (continuous), and phenotypic index (categories 0-4).

[c]HRs by Breslow category estimated in Cox proportional hazards models adjusted for age (continuous), sex, study centre, whether a first or higher-order primary melanoma, log of Breslow thickness, site (head/neck, trunk, arms, legs), and phenotypic index (categories 0-4).

[d]HRs by primary status estimated in Cox proportional hazards models adjusted for age (continuous), sex, study centre, log of Breslow thickness, site (head/neck, trunk, arms, legs), and phenotypic index (categories 0-4).

[e]$P_{interaction}$ calculated using a log-likelihood test comparing the multivariable-adjusted model with and without the interaction term.

Figure Legends

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 ≤2.0 mm thick tumors (n = 3679), and (**C**) participants with >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.

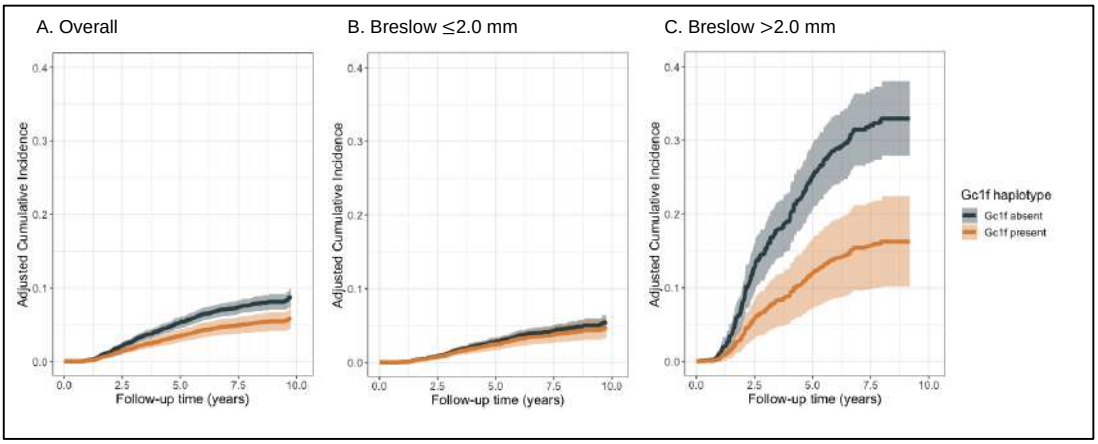

Figure 1