Title

Association of Incident Amelanotic Melanoma With Phenotypic Characteristics, MC1R Status, and Prior Amelanotic Melanoma

Journal

JAMA Dermatology, 153(10)

ISSN

2168-6068

Authors

Vernali, Steven
Waxweiler, Weston T
Dillon, Patrick M
et al.

Publication Date

2017-10-01

DOI

10.1001/jamadermatol.2017.2444

Peer reviewed

HHS Public Access

Author manuscript

JAMA Dermatol. Author manuscript; available in PMC 2018 October 01.

Published in final edited form as:

JAMA Dermatol. 2017 October 01; 153(10): 1026–1031. doi:10.1001/jamadermatol.2017.2444.

Association of Incident Amelanotic Melanoma with Phenotypic Characteristics, *MC1R* Status, and Prior Amelanotic Melanoma

Steven Vernali, BA, **Weston T. Waxweiler, MD**, **Patrick M. Dillon, MD**, **Peter A. Kanetsky, MPH, PhD**, **Irene Orlow, PhD**, **Li Luo, PhD**, **Klaus J. Busam, MD**, **Anne Kricker, PhD**, **Bruce K. Armstrong, MBBS, DPhil**, **Hoda Anton-Culver, PhD**, **Stephen B. Gruber, MD, MPH, PhD**, **Richard P. Gallagher, MA**, **Roberto Zanetti, MD**, **Stefano Rosso, MD, MSc**, **Lidia Sacchetto, MSc**, **Terence Dwyer, MD**, **Anne E. Cust, PhD**, **David W. Ollila, MD**, **Colin B. Begg, PhD**, **Marianne Berwick, MPH, PhD**, and **Nancy E. Thomas, MD, PhD on behalf of the GEM Study Group**

Department of Dermatology, University of North Carolina, Chapel Hill, NC (SV, WTW, NET); Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC (NET, DWO); Department of Medicine, University of Virginia, Charlottesville, VA (PMD), Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL (PAK); Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, NY (IO, KJB, CBB); Department of Surgery, University of North Carolina, Chapel Hill, NC (DWO); Department of Internal Medicine, University of New Mexico Cancer Center, University of New Mexico, Albuquerque, NM (LL, MB); Sydney School of Public Health, University of Sydney, Sydney, New South Wales, Australia (AEC, AK, BKA); Department of Epidemiology, University of California, Irvine, CA (HAC); USC Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA (SBG); Cancer Control Research, British Columbia Cancer Agency, Vancouver, British Columbia, Canada (RPG); Piedmont Cancer Registry, Centre for Epidemiology and Prevention in Oncology in Piedmont, Turin, Italy (RZ, SR, LS); Politecnico di Torino, Turin, Italy (LS); Universitá degli Studi di Torino, Turin, Italy (LS); George Institute for Global Health, Nuffield Department of Obstetrics and Gynecology, University of Oxford (TD)

Abstract

Correspondence to: Nancy E. Thomas, MD PhD, Department of Dermatology, University of North Carolina, 2159 Genomic Science Bldg., CB#7287, Chapel Hill, NC 27599; Phone: (919) 966-0785; nancy_thomas@med.unc.edu.

Financial Disclosure: None reported.

Author Contributions: Dr. Nancy E. Thomas and Mr. Steven Vernali had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design*: Thomas, Kanetsky, Orlow, Berwick. *Acquisition, analysis, and interpretation of data*: Thomas, Vernali, Waxweiler, Dillon, Kanetsky, Orlow, Luo, Berwick. *Drafting of the manuscript*: Vernali, Thomas, Waxweiler, Dillon, Kanetsky, Orlow, Luo, Berwick. *Critical revision of the manuscript for important intellectual content*: Vernali, Thomas, Waxweiler, Dillon, Kanetsky, Orlow, Luo, Busam, Kricker, Armstrong, Anton-Culver, Gruber, Gallagher, Zanetti, Rosso, Sacchetto, Dwyer, Cust, Ollila, Begg, Berwick, Thomas. *Statistical analysis*: Vernali, Thomas, Waxweiler, Kanetsky, Orlow, Luo, Berwick. Obtained funding: Thomas, Berwick, Begg, Armstrong, Cust. *Administrative, technical, or material support*: Thomas, Berwick, Armstrong, Anton-Culver, Gruber, Gallagher, Zanetti, Dwyer. *Study supervision*: Thomas.

Funding/Sponsor was involved? NO
Design and conduct of the study? NO
Collection, management, analysis, and interpretation of data? NO
Preparation, review, or approval of the manuscript? NO
Decision to submit the manuscript for publication? NO

Importance—We previously reported that survival is poorer from histopathologically amelanotic than pigmented melanoma because of more advanced stage at diagnosis. Identifying patients at risk of amelanotic melanoma might enable earlier diagnosis and improved survival; however, the phenotypic characteristics and underlying genetics associated with amelanotic melanoma are unknown.

Objective—To determine whether phenotypic characteristics, carriage of *MC1R* variants, and history of amelanotic melanoma are associated with histopathologically amelanotic melanoma.

Design—The Genes, Environment, and Melanoma (GEM) study is an international study that enrolled patients with incident primary cutaneous melanomas from 1998–2003.

Setting—Cases ascertained from population-based and hospital-based cancer registries.

Participants—The GEM participants included here were 2387 patients with data for phenotypes, *MC1R* genotype, and primary melanomas scored for histopathologic pigmentation. Of these 2387 patients with incident melanomas scored for pigmentation, 527 had prior primary melanomas also scored for pigmentation.

Main Outcome and Measures—Associations of phenotypic characteristics (freckles, nevi, phenotypic index) and *MC1R* status with incident amelanotic melanomas were evaluated using logistic regression models adjusted for age, sex, study center, and primary status (single or multiple primary melanoma); ORs and 95% CIs are reported. Association of histopathologic pigmentation between incident and prior melanomas was analyzed using an exact logistic regression model.

Results—In a multivariable model including phenotypic characteristics, absence of back nevi, presence of many freckles, and a sun-sensitive phenotypic index were independently associated with amelanotic melanoma (each $P < .05$). Carriage of *MC1R* variants was associated with amelanotic melanoma, but lost statistical significance in a model with phenotype. Further, patients with incident primary amelanotic melanomas were more likely to have had a prior primary amelanotic melanoma (OR = 4.62, 95% CI = 1.25–14.13) than those with incident primary pigmented melanomas.

Conclusions and Relevance—Absence of back nevi, presence of many freckles, a sun-sensitive phenotypic index, and prior amelanotic melanoma increase odds for development of amelanotic melanoma. An increased index of suspicion for melanoma in presenting non-pigmented lesions and more careful examination for signs of amelanotic melanoma during periodic skin examination in patients at increased odds of amelanotic melanoma might lead to earlier diagnosis and improved survival.

Introduction

Amelanotic melanoma is defined as melanoma without pigment on inspection[1] or lacking melanin on histopathologic examination.[2] Approximately 2–8% of melanomas are amelanotic.[3] In the international, population-based, Genes, Environment, and Melanoma (GEM) study, we reported that survival is poorer from amelanotic than pigmented melanoma due to more advanced stage at diagnosis.[2] Studies examining patient characteristics associated with amelanotic melanoma have been limited to demographic and genotypic

descriptions.[1,4–7] Amelanotic melanoma was associated with older age in GEM[2] and other studies,[1,4] and predominantly found in Caucasians.[1,4] Associations with sex have been less consistent as previously discussed for GEM.[2] Patients with amelanotic melanoma have been reported to carry *MC1R* variants linked to red hair ('R')[5] and/or *MITF* E318K.[6] A study of 118 melanomas found *MC1R* 'R' variants positively associated with amelanotic cases.[7] Our goal was to compare phenotype, *MC1R* status, and history of amelanotic melanoma between amelanotic and pigmented melanoma patients.

Methods

Population

The GEM study included 3579 patients with incident primary cutaneous melanoma from 1998–2003 at eight sites in Australia, Canada, Italy, and the United States.[8] Institutional review boards at each center reviewed and approved the study. Patients gave written, informed consent. GEM ascertained data for incident (index) and prior melanomas from cancer registries. This report includes 2387 (66.7% of 3579) GEM participants with data for phenotype, *MC1R* genotyping, and melanomas scored for histopathologic pigmentation. Of these 2387 patients with incident melanomas scored for pigmentation, 527 had prior primary melanomas also scored for histopathologic pigmentation. According to GEM protocol, *in situ* melanomas were incident melanomas if patients had prior invasive melanomas.

Self-administered questionnaires and telephone interviews were used to ascertain melanoma risk factors.[8] Back nevi were counted by family using glossy colored guides to aid nevus identification.[8] *MC1R* was sequenced from DNA from buccal swabs.[9] Variants were classified by strength of association with red hair as in Taylor et al. ("R": D84E, R142H, R151C, R160W, and D294H, all nonsense and insertion/deletion; "r": all other variants; "wt": consensus).[10] Histopathologic pigmentation was determined by observation of melanin granules on light microscopy during centralized review of diagnostic slides for both index and prior primary melanomas.[2] We previously reported that histopathologic pigmentation scoring had moderate interobserver agreement (kappa=0.48) and a significant association with clinical, pre-biopsy impression of pigmentation from pathology reports.[2] A scalar phenotypic index was derived by combining hair color, eye color, and ability to tan as previously described.[11] This index was dichotomized to indicate sun-resistant (scores of 0, 1, or 2) and sun-sensitive (scores of 3, 4, or 5) phenotypes.

Statistical Analysis

Among participants with incident single primary melanoma (SPM) or multiple primary melanoma (MPM), we estimated the odds ratios (OR) and 95% confidence intervals (95% CI) for associations of phenotypic characteristics (freckles, nevi, phenotypic index) and *MC1R* status using logistic regression models adjusted for study design features: age, sex, study center, and lesion status (SPM or index MPM). Multivariable models were developed including the three phenotypic characteristics alone or with *MC1R* status to identify factors independently associated with amelanotic melanoma. The same analyses with individual phenotypic characteristics and separated *MC1R* genotypes were also performed. Association of histopathologic pigmentation between incident and prior melanomas for patients with

MPM was analyzed using exact logistic regression. Association with *MITF* E318K mutations was also analyzed using exact logistic regression. Statistical tests were two-sided with $P < .05$ considered significant. Data were analyzed using STATA version 13 (Stata-Corp LP, College Station, TX).

Results

Overall, 178 (7.5% of 2387) incident and 32 (6.1% of 527) prior primary melanomas were amelanotic (Table 1).

Phenotypic Characteristics and *MC1R* Variants

In 2387 participants with incident melanomas (Table 2), absence of back nevi (OR = 1.76, 95% CI = 1.18–2.65; $P = .006$), presence of many freckles (OR = 1.76, 95% CI = 1.17–2.65; $P = .007$), a sun-sensitive phenotypic index (OR = 1.57, 95% CI = 1.14–2.18; $P = .006$), and carriage of *MC1R* variants (OR = 1.70, 95% CI = 1.04–2.78 for r/r, R/r, or R/R genotypes; P for trend = .01) were associated with amelanotic melanoma, adjusting for study design features.

Including the phenotypic characteristics in one multivariable model, absence of back nevi (OR = 1.71, 95% CI = 1.14–2.57; $P = .01$), presence of many freckles (OR = 1.58, 95% CI = 1.04–2.39; $P = .03$), and a sun-sensitive phenotypic index (OR = 1.48, 95% CI = 1.07–2.07; $P = .02$) were significantly associated with amelanotic melanoma.

Adding *MC1R* to the multivariable model of phenotypic characteristics, absence of back nevi (OR = 1.68, 95% CI = 1.12–2.53; $P = .01$) and a sun-sensitive phenotypic index (OR = 1.44, 95% CI = 1.03–2.01; $P = .03$) remained statistically significant, but not presence of many freckles or *MC1R*. Attenuation of the association with *MC1R* was explained by the addition of freckles and phenotypic index to the model. Despite the attenuation, the point estimate for the r/r, R/r, R/R variants was similar to that for freckling and phenotypic index.

Results for individual phenotypic characteristics and separate *MC1R* genotypes are in Table S1. Table S2 shows *MITF* E318K was not associated with amelanotic melanoma in our study (OR = 0.86, 95% CI = 0.22–2.37; $P = .82$).

Incident and Prior Melanoma Pigmentation

Table 3 shows associations of incident amelanotic melanoma with the pigmentation state of the previous melanoma in 527 MPM participants with pigmentation scored for each melanoma. Of 24 patients with incident amelanotic melanomas, 5 (20.8%) had prior amelanotic melanomas. For 503 patients with incident pigmented melanomas, 27 (5.4%) had prior amelanotic melanomas. Patients with an incident amelanotic melanoma were more likely to have a prior amelanotic melanoma than those with an incident pigmented melanoma (OR = 4.62, 95% CI = 1.25–14.13; $P = .01$).

Discussion

Our findings suggest that patients with prior amelanotic melanomas remain at risk of pigmented melanoma, but have increased odds of developing subsequent amelanotic melanomas. Further, we found independent associations of absence of back nevi, presence of many freckles, and a sun-sensitive phenotypic index with amelanotic melanoma. *MC1R*, a genetic determinant of phenotype (especially freckling and red hair),[9] was also associated with amelanotic melanoma. This association lost statistical significance in a model with phenotype, but the point estimate for the r/r, R/r, R/R variants was similar to that for the correlated phenotype variables. Thus, the association of *MC1R* with amelanotic melanoma may not be entirely accounted for by phenotype.

Although clinicians may expect that patients with sun-sensitive phenotypes or history of amelanotic melanoma are more likely to develop amelanotic melanoma, we are unaware of another study examining amelanotic melanoma's associations with phenotype or prior amelanotic melanoma. One report consistent with GEM described three amelanotic melanomas in a patient with red hair, fair skin, many freckles, and few nevi.[6] Also similar to GEM, Ghiorzo et al.[7] found an association of *MC1R* with amelanotic melanoma.

Strengths of our study include the large, international, population-based study design; centralized dermatopathology review; and objective definition of pigmentation. A limitation is that melanoma pigmentation may be misclassified due to interobserver variability. While we did not have pre-biopsy pigmentation, we previously reported that the clinical, pre-biopsy impression of pigmentation extracted from pathology reports was significantly associated with histopathologic pigmentation in a subset of GEM patients.[2]

Conclusions

Increased index of suspicion for melanoma in presenting non-pigmented lesions and careful periodic screening for signs of amelanotic and pigmented melanoma in patients at increased odds of amelanotic melanoma might lead to earlier diagnosis and improved survival. Dermoscopy and confocal microscopy, useful for diagnosis of amelanotic melanoma,[12,13] could be helpful. Research to determine whether other genetic polymorphisms associated with pigmentary characteristics and/or nevi[14,15] are associated with amelanotic melanoma is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding/Support: This study was supported in part by the National Cancer Institute (R01CA112243 to N.E.T, U01CA83180 and R01CA112524 to M.B., R01CA098438 to C.B.B, P30CA016086 to Norman E. Sharpless, P30CA014089 to S.B.G, and P30CA008748 to Craig B. Thompson); National Institute of Environmental Health Sciences (P30ES010126 to James A. Swenberg); and University of Sydney Medical Foundation Program grant to B.K.A. A.E.C received fellowships from the NHMRC and Cancer Institute NSW.

We are indebted to the GEM Study Group: Coordinating Center, Memorial Sloan Kettering Cancer Center, New York, NY (USA): Marianne Berwick (PI, currently at the University of New Mexico), Colin Begg, Ph.D. (co-PI), Irene Orlow, Ph.D., M.S. (co-Investigator), Klaus J. Busam, M.D. (Dermatopathologist), Pampa Roy, Ph.D. (Senior Laboratory Technician), Himali Patel, M.S. (Senior Laboratory Technician); University of New Mexico, Albuquerque: Marianne Berwick, M.P.H., Ph.D. (PI), Li Luo, Ph.D. (Biostatistician), Susan Paine, 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 (current PI), Terence Dwyer (PI, currently at University of Oxford, United Kingdom), Paul Tucker (Dermatopathologist); British Columbia Cancer Research Centre, Vancouver, Canada: Richard P. Gallagher, M.A. (PI), Cancer Care Ontario, Toronto, Canada: Loraine D. Marrett, Ph.D. (PI), Lynn From, M.D. (Dermatopathologist); CPO, Center for Cancer Prevention, Torino, Italy: Roberto Zanetti, M.D (PI), Stefano Rosso, M.D., M.Sc. (co-PI); University of California, Irvine, CA: Hoda Anton-Culver, Ph.D. (PI); University of Michigan, Ann Arbor, MI: University of Michigan, Ann Arbor: Stephen B. Gruber, M.D., M.P.H., Ph.D. (PI, currently at University of Southern California, Los Angeles, CA), Shu-Chen Huang, M.S., M.B.A. (co-Investigator, joint at USC-University of Michigan); New Jersey Department of Health and Senior Services, Trenton, NJ ; University of North Carolina, Chapel Hill, NC: Nancy E. Thomas, M.D., Ph.D. (PI), David W. Ollila, M.D. (co-Investigator), Pamela A. Groben, M.D. (Dermatopathologist), David C. Gibbs, B.S. (Research Assistant); 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, Florida); UV data consultants: Julia Lee Taylor, Ph.D. and Sasha Madronich, Ph.D., National Centre for Atmospheric Research, Boulder, CO.

References

1. McClain SE, Mayo KB, Shada AL, Smolkin ME, Patterson JW, Slingluff CL Jr. Amelanotic melanomas presenting as red skin lesions: a diagnostic challenge with potentially lethal consequences. Int J Dermatol. 2012; 51(4):420–426. [PubMed: 22435430]
2. Thomas NE, Kricker A, Waxweiler WT, et al. Comparison of Clinicopathologic Features and Survival of Histopathologically Amelanotic and Pigmented Melanomas: A Population-Based Study. JAMA Dermatol. 2014; 150(12):1306–1314. [PubMed: 25162299]
3. Gualandri L, Betti R, Crosti C. Clinical features of 36 cases of amelanotic melanomas and considerations about the relationship between histologic subtypes and diagnostic delay. J Eur Acad Dermatol Venereol. 2009; 23(3):283–287. [PubMed: 19207640]
4. Moreau JF, Weissfeld JL, Ferris LK. Characteristics and survival of patients with invasive amelanotic melanoma in the USA. Melanoma Res. 2013; 23(5):408–413. [PubMed: 23883947]
5. Zalaudek I, Meiklejohn W, Argenziano G, Thurber AE, Sturm RA. “White” nevi and “red” melanomas: association with the RHC phenotype of the MC1R gene. J Invest Dermatol. 2009; 129(5):1305–1307. [PubMed: 19052562]
6. Sturm RA, Fox C, McClenahan P, et al. Phenotypic characterization of nevus and tumor patterns in MITF E318K mutation carrier melanoma patients. J Invest Dermatol. 2014; 134(1):141–149. [PubMed: 23774529]
7. Ghiorzo P, Pastorino L, Pizzichetta MA, et al. CDKN2A and MC1R analysis in amelanotic and pigmented melanoma. Melanoma Res. 2009; 19(3):142–145. [PubMed: 19339902]
8. Begg CB, Hummer AJ, Mujumdar U, et al. 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. [PubMed: 16556646]
9. Kanetsky PA, Rebbeck TR, Hummer AJ, et al. Population-based study of natural variation in the melanocortin-1 receptor gene and melanoma. Cancer Res. 2006; 66(18):9330–9337. [PubMed: 16982779]
10. Taylor NJ, Busam KJ, From L, et al. Inherited variation at MC1R and histological characteristics of primary melanoma. PLoS One. 2015; 10(3):e0119920. [PubMed: 25790105]
11. Gibbs DC, Orlow I, Kanetsky PA, et al. Inherited genetic variants associated with occurrence of multiple primary melanoma. Cancer Epidemiol Biomarkers Prev. 2015; 24(6):992–997. [PubMed: 25837821]
12. Guitera P, Pellacani G, Crotty KA, et al. The impact of in vivo reflectance confocal microscopy on the diagnostic accuracy of lentigo maligna and equivocal pigmented and nonpigmented macules of the face. J Invest Dermatol. 2010; 130(8):2080–2091. [PubMed: 20393481]

13. Pizzichetta MA, Kittler H, Stanganelli I, et al. Dermoscopic diagnosis of amelanotic/hypomelanotic melanoma. Br J Dermatol. 2016

14. Duffy DL, Zhao ZZ, Sturm RA, Hayward NK, Martin NG, Montgomery GW. Multiple pigmentation gene polymorphisms account for a substantial proportion of risk of cutaneous malignant melanoma. J Invest Dermatol. 2010; 130(2):520–528. [PubMed: 19710684]

15. Han J, Kraft P, Nan H, et al. A genome-wide association study identifies novel alleles associated with hair color and skin pigmentation. PLoS Genet. 2008; 4(5):e1000074. [PubMed: 18483556]

Key Points

Question

Are phenotypic characteristics, *MC1R* variants, and prior amelanotic melanoma associated with amelanotic melanoma?

Findings

Absence of back nevi, presence of many freckles, a sun-sensitive phenotype, and prior amelanotic melanoma were associated with development of amelanotic melanoma. *MC1R* was associated with amelanotic melanoma but this association lost significance in a model with phenotype.

Meaning

Prior amelanotic melanoma and the phenotypes associated with it should raise clinicians' index of suspicion for amelanotic melanoma when examining a suspicious but non-pigmented skin lesion, and clinicians might also use these characteristics to prompt periodic, meticulous screening of non-pigmented as well as pigmented skin lesions.

Table 1

Characteristics of 2914 Primary Melanomas from 2387 Patients Scored for Histopathologic Pigmentation in the GEM Study

	Incident Primary Melanoma	Prior Primary Melanoma
Characteristic	**(n=2387)[a]**	**(n=527)[a]**
Sex		
Male	1322 (55.4)	354 (67.2)
Female	1065 (44.6)	173 (32.8)
Age at diagnosis, y		
Mean (±SD)	58.3 ± 16.1	65.9 ± 12.9
< 50	712 (29.8)	62 (11.8)
50–69	966 (40.5)	222 (42.1)
≥ 70	709 (29.7)	243 (46.1)
Race/Ethnicity		
Caucasian	2380 (99.7)	526 (99.8)
Non-Caucasian	7 (0.3)	1 (0.2)
Country		
Australia (New South Wales & Tasmania)	1096 (45.9)	394 (74.8)
Canada (British Columbia & Ontario)	547 (22.9)	76 (14.4)
Italy (Torino)	75 (3.1)	2 (0.4)
United States (NC, NJ, MI, and CA)	669 (28.0)	55 (10.4)
Histopathologic pigmentation		
Pigmented	2209 (92.5)	495 (93.9)
Amelanotic	178 (7.5)	32 (6.1)
Histologic subtype		
Superficial Spreading	1551 (65.0)	353 (67.0)
Nodular	182 (7.6)	40 (7.6)
Lentigo maligna	286 (12.0)	87 (16.5)
In-situ	161 (6.7)	0 (0.0)
Unclassified/other[b]	207 (8.7)	47 (8.9)
Anatomic Site		
Head, neck	437 (18.3)	84 (15.9)
Trunk, pelvis	1040 (43.6)	248 (47.1)
Upper extremities	424 (17.8)	94 (17.8)
Lower extremities	486 (20.4)	101 (19.2)
Breslow thickness, mmc	(n=2,380)	(n=525)
Median (IQR), mm	0.6 (0.8)	0.7 (0.7)
In-situ	167 (7.0)	0 (0.0)
0.01 to 1.00	1518 (63.8)	365 (69.5)

	Incident Primary Melanoma	Prior Primary Melanoma
Characteristic	**(n=2387)[a]**	**(n=527)[a]**
1.01 to 2.00	405 (17.0)	95 (18.1)
2.01 to 4.00	195 (8.2)	49 (9.3)
>4.00	95 (4.0)	16 (3.1)

Abbreviations: GEM = Genes, Environment, and Melanoma; IQR = interquartile range; SD = standard deviation.

[a] Data are given as number (percentage) of melanomas.

[b] Other includes acral lentiginous, spindle cell, nevoid, and Spitzoid melanomas.

[c] Counts do not sum to the total number of study subjects due to missing data.

Table 2

Phenotypic Characteristics and MC1R Status in Relationship to Histopathologic Pigmentation in Incident Primary Melanomas from 2387 patients in the GEM Study

			Amelanotic vs. Pigmented Melanoma					
	Pigmented Melanoma	Amelanotic Melanoma	Study Design Features[b]		Study Design Features + Phenotype[c]		Study Design Features + Phenotype + MC1R[d]	
Characteristics	**(n = 2209)[a]**	**(n = 178)[a]**	**OR (95% CI)**	***P* Value[e]**	**OR (95% CI)**	***P* Value[e]**	**OR (95% CI)**	***P* Value[e]**
Phenotype								
Back nevi								
Present	1904 (86.2)	139 (78.1)	1 [Reference]	0.006	1 [Reference]	0.01	1 [Reference]	0.01
Absent	305 (13.8)	39 (21.9)	1.76 (1.18–2.65)		1.71 (1.14–2.57)		1.68 (1.12–2.53)	
Freckles								
None to few	1912 (86.6)	143 (80.3)	1 [Reference]	0.007	1 [Reference]	0.03	1 [Reference]	0.10
Many	297 (13.4)	35 (19.7)	1.76 (1.17–2.65)		1.58 (1.04–2.39)		1.44 (0.93–2.22)	
Phenotypic index[f]								
Sun-resistant phenotypic index	989 (47.1)	68 (38.2)	1 [Reference]	0.006	1 [Reference]	0.02	1 [Reference]	0.03
Sun-sensitive phenotypic index	1109 (52.9)	110 (61.8)	1.57 (1.14–2.18)		1.48 (1.07–2.07)		1.44 (1.03–2.01)	
Genetics								
MC1R[g]								
wt/wt	357 (16.2)	23 (12.9)	1 [Reference]		-	-	1 [Reference]	
r/wt or R/wt	980 (44.4)	69 (38.8)	1.17 (0.71–1.93)		-	-	1.16 (0.70–1.92)	
r/r, R/r, or R/R	872 (39.5)	86 (48.3)	1.70 (1.04–2.78)		-	-	1.43 (0.86–2.39)	
P value for trend				0.01	-	-		0.13

Abbreviations: CI, confidence interval; GEM, genes, environment, and melanoma; MC1R, melanocortin 1 receptor; MPM, multiple primary melanoma; OR, odds ratio; SPM, single primary melanoma.

[a] Data are given as number (percentage) of melanomas unless otherwise specified.

[b] Adjusted for study design features: sex, age, study center, and lesion status (SPM or index MPM)

[c] Adjusted for study design features, freckling, back moles, and phenotypic index.

[d] Adjusted for study design features and all characteristics displayed in the table.

[e] *P* values were calculated according to logistic regression models and *P* values for trend were calculated including MC1R categories as ordinal variables.

[f] Phenotypic index was calculated by additively combining: hair color (black or dark brown=0; light brown or blonde=1; red=2), eye color (brown=0; grey, green, or hazel=1; blue=2), and ability to tan (deeply or moderately=0; occasionally or none=1). Those with index scores of 0,1, or 2 were categorized as having a sun-resistant phenotypic index. Patients with index scores of 3, 4, or 5 were categorized as having a sun-sensitive phenotypic index.

[g] *MC1R* "R": D84E, R142H, R151C, R160W, and D294H, all nonsense and insertion/deletion; "r": all other variants; "wt": consensus.

Table 3

Association of Histopathologic Pigmentation Between Incident and Prior Melanomas in 527 Multiple Primary Melanoma Patients in the GEM Study[a]

	Incident Primary Melanoma			
	Pigmented Melanoma	**Amelanotic Melanoma**	**Amelanotic vs. Pigmented Prior Primary Melanoma**	
Prior Primary Melanoma	n (%)[b]	n (%)[b]	OR (95%CI)	*P* Value[c]
Pigmented Melanoma	476 (94.6)	19 (79.2)	1 [Reference]	0.01
Amelanotic Melanoma	27 (5.4)	5 (20.8)	4.62 (1.25–14.13)	

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Includes only patients with second or higher order primary melanomas scored for histopathologic pigmentation

[b] Data are given as number (percentage) of melanomas.

[c] *P* value was calculated using an exact logistic regression model.