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Associations of *MC1R* genotype and patient phenotypes with *BRAF* and *NRAS* mutations in melanoma

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Abstract

Associations of *MC1R* with *BRAF* mutations in melanoma have been inconsistent between studies. We sought to determine for 1227 participants in the international population-based Genes, Environment and Melanoma (GEM) study whether *MC1R* and phenotypes were associated with melanoma *BRAF/NRAS* subtypes. We used logistic regression adjusted by age, sex, and study design features and examined effect modifications. *BRAF*+ were associated with younger age, blond/light brown hair, increased nevi, and less freckling and *NRAS*+ with older age relative to WT (*BRAF*−/*NRAS*−) melanomas (all $P<0.05$). Comparing specific *BRAF* subtypes to WT, *BRAF* V600E was associated with younger age, blond/light brown hair, and increased nevi and V600K with increased nevi and less freckling (all $P<0.05$). *MC1R* was positively associated with *BRAF* V600E cases but only among individuals with darker phototypes or darker hair ($P_{interaction}<0.05$), but inversely associated with *BRAF* V600K ($P_{trend}=0.006$) with no significant effect modification by phenotypes. These results support distinct etiologies for *BRAF* V600E, *BRAF* V600K, *NRAS*+, and WT melanomas. *MC1R*'s associations with *BRAF* V600E cases limited to individuals with darker phenotypes indicate that *MC1R* genotypes specifically provide information about *BRAF* V600E melanoma risk in those not considered high risk based on phenotype. Our results also suggest melanin pathways deserve further study in *BRAF* V600E melanomagenesis.

Keywords

Melanoma; risk; population-based; pigmentation; sunburn; *MC1R*; genotype; sun exposure; dermatology; epidemiology; melanin; melanocytes; nevus; hair color; eye color

INTRODUCTION

Individual risk of melanoma depends on constitutional and environmental factors. Patients' age, tannability, nevus numbers, freckling, and UV exposure have been associated with melanoma molecular subtypes, particularly those defined by somatic driver mutations in *BRAF* and *NRAS* (Hacker et al., 2010, Hacker et al., 2013, Hacker et al., 2016, Liu et al., 2007, Thomas et al., 2007, Wu et al., 2014). *MC1R*, the melanocortin 1 receptor, controls melanin production, pigmentation phenotypes, and influences sun sensitivity and melanoma risk (Wolf Horrell et al., 2016). *MC1R* germline variants were associated with *BRAF*+ melanomas in studies in Italy and San Francisco (Fargnoli et al., 2008, Landi et al., 2006) but not in North Carolina or Australia (Hacker et al., 2010, Hacker et al., 2016, Thomas et al., 2010b), while the association was restricted to head and neck melanomas in a study from Spain and Austria (Hacker et al., 2013). Further, *BRAF* V600K tumors were more common in men and at older ages and had more adjacent solar elastosis than V600E tumors (Bucheit et al., 2013, Jewell et al., 2012, Menzies et al., 2012). Patients with *BRAF* V600K tumors had similarly high nevus counts as V600E cases (Hacker et al., 2016). The associations of *MC1R* and phenotypes with *BRAF/NRAS* status in melanoma have been restricted to studies which had <500 participants (Fargnoli et al., 2008, Garcia-Casado et al., 2015, Hacker et al., 2010, Hacker et al., 2013, Landi et al., 2006, Liu et al., 2007, Scherer et al., 2010, Wu et al., 2014), including initial reports of subsets of melanomas from the Genes, Environment, and Melanoma (GEM) Study (Poynter et al., 2006, Thomas et al., 2007, Thomas et al., 2010b).

The GEM study included 1227 melanoma patients with tumor *BRAF/NRAS* mutational status, patient phenotypes, and tumor characteristics. Information on *MC1R* germline sequence was available for 1044 of these participants. The purpose was to describe associations of *MC1R* risk variants, phenotypes, and tumor characteristics with *BRAF/NRAS* subtypes in this large international population-based study.

RESULTS

Subject Characteristics

The 1227 GEM patients with tumors analyzed for *BRAF* and *NRAS* mutations had a median age of 60.0 years; 59.4% were male; 61.4% were from Australia and 38.6% from the United States (Table 1). These 1227 melanomas (all from different patients) were from 912 patients (74.3%) who had only one melanoma at the time of recruitment (a first primary melanoma) and 315 patients (25.7%) who had more than one melanoma at the time of recruitment. For the latter group of patients, we retrieved and utilized for these analyses the 315 second or higher order primary occurring at the time of recruitment. The melanomas were 26.3% *BRAF*+, 13.5% *NRAS*+, and 60.2% WT (*BRAF*−/*NRAS*−). The majority of *BRAF*+ melanomas were *BRAF* V600E (68.1%); 21.1% were V600K, and 10.8% were *BRAF* other. *BRAF* and *NRAS* mutations were mutually exclusive. The predominant subtype was superficial spreading melanoma (SSM) (67.9%). The median Breslow thickness was 0.70 mm.

Relationship of Tumor Characteristics, Phenotypes, and *MC1R* to *BRAF/NRAS* subtypes

We compared *BRAF*+ and *NRAS*+ tumors separately to WT and then *BRAF*+ to *NRAS*+ regarding tumor characteristics (Table 2). Lentigo maligna melanomas (LMM) were less likely to harbor *BRAF* mutations than SSM, as were unclassified/other types ($P < 0.001$). Nodular melanomas (NM) were more likely and LMM less likely than SSM to carry *NRAS* mutations ($P < 0.001$). *BRAF*+ tumors were associated with Breslow thickness 1.01–2.00 vs. 0.01–1.00 mm, truncal site, present neval remnants, and absent solar elastosis (all $P < 0.05$). *NRAS*+ tumors were associated with increased Breslow thickness and absent solar elastosis (both $P < 0.05$). *NRAS*+ tumors were less likely on the head/neck than trunk ($P < 0.001$). Compared with *NRAS*+, *BRAF*+ tumors were less likely to be NM or unclassified/other than SSM, less likely on the upper extremities than trunk, and tended to be thinner (all $P < 0.05$).

Compared with WT, younger age was associated with *BRAF*+ and older age with *NRAS*+ melanoma (both $P_{trend} < 0.05$) (Table 3). Blond/light brown, but not red, compared to dark brown/black hair and fewer freckles were associated with *BRAF*+ melanoma (both $P < 0.05$). Self-reported back nevi were associated with *BRAF*+ ($P_{trend} = 0.02$) and *NRAS*+ tumors but with $P_{trend} = 0.09$. *MC1R* status was not significantly associated with *BRAF*+ or *NRAS*+ compared with WT tumors. Relative to *NRAS*+, younger age and blond/light brown hair were associated with *BRAF*+ tumors (both $P < 0.05$).

There were no significant interactions on the relationship of *MC1R* with *BRAF*+ compared to WT melanoma by solar elastosis (absent versus present or severe versus absent/mild/moderate) or site (head/neck versus other) (data not shown).

Relationship of Tumor Characteristics, Phenotypes, and *MC1R* to Specific *BRAF* Subtypes

We next compared *BRAF* V600E and V600K tumors separately to WT and then V600K to V600E regarding tumor characteristics (Table 4). LMMs and unclassified/other types were less likely than SSM to carry *BRAF* V600E or V600K mutations (both $P < 0.05$). *BRAF* V600E was associated with truncal site, Breslow thickness 1.01–2.00 vs. 0.01–1.00 mm, present neval remnants, and absent solar elastosis. (all $P < 0.05$). Compared with *BRAF* V600E, V600K was associated with head/neck site, absent neval remnants, and present solar elastosis (all $P < 0.05$).

Compared with WT melanoma, younger age and increased back nevi were associated with *BRAF* V600E (both $P_{trend} < 0.05$) (Table 5). Blond/light brown, but not red, relative to dark brown/black hair was positively associated with *BRAF* V600E melanoma ($P = 0.03$). There was a non-significant positive association of *MC1R* with *BRAF* V600E melanoma ($P_{trend} = 0.08$). Increased back nevi and fewer freckles were associated with *BRAF* V600K melanoma (all $P_{trend} < 0.05$). Patients with *MC1R* variants were less likely to have *BRAF* V600K than WT melanomas (OR = 0.34, 95% CI = 0.17–0.66 for R/R, R/r, or R/wt vs. wt/wt; $P_{trend} = 0.006$). Compared with V600E, older age and fewer freckles were associated with *BRAF* V600K tumors (both $P_{trend} < 0.05$). Patients with *MC1R* variants were less likely to have *BRAF* V600K than V600E tumors (OR = 0.24, 95% CI = 0.11–0.55 for R/R, R/r, or R/wt vs. wt/wt; $P_{trend} = 0.002$).

In re-analyses also adjusted for tannability, hair color, eye color, back nevi, and freckling (Supplementary Table 1, available online), the association of *MC1R* variants strengthened for *BRAF* V600E compared with WT tumors (OR = 1.66, 95% CI = 0.90–3.07 for R/R, R/r, or R/wt vs. wt/wt; P_{trend} = 0.03). Patients with *MC1R* variants remained significantly less likely to have *BRAF* V600K than WT tumors (OR = 0.36, 95% CI = 0.17–0.77 for R/R, R/r, or R/wt vs. wt/wt; P_{trend} = 0.02) or *BRAF* V600K than V600E tumors (OR = 0.14, 95% CI = 0.05–0.42 for R/R, R/r, or R/wt vs. wt/wt; P_{trend} < 0.001). There were no significant interactions on the relationship of *MC1R* with *BRAF* subtypes compared with WT by center or first versus higher order primary melanoma in these analyses (data not shown).

Effect modifications by phenotypes on the associations of *MC1R* with *BRAF* subtypes

Due to inconsistent results in prior publications and suggestions in the literature that tanning response or pigmentation could influence the associations of *MC1R* with *BRAF* melanoma (Thomas et al., 2010a), we examined effect modification by phenotypes (Table 6). We found evidence that the association of *MC1R* with *BRAF* V600E (compared with WT) was modified by tannability, tendency to burn, and hair color (each $P_{interaction}$ < 0.05) and also by eye color ($P_{interaction}$ = 0.06) but not by back nevi ($P_{interaction}$ = 0.83) or freckling ($P_{interaction}$ = 0.20). *MC1R* was significantly associated with *BRAF* V600E compared with WT tumors among patients with increased tannability, decreased tendency to burn, darker hair, and darker eyes (all P_{trend} < 0.05) but not among those with fairer phenotypes. There was no evidence of effect modification by phenotype on the association of *MC1R* with *BRAF* V600K compared with WT. The association of *MC1R* with *BRAF* V600K compared with V600E was modified by tendency to burn ($P_{interaction}$ = 0.01) but not by other phenotypes that could be analyzed. Some interactions could not be examined due to insufficient sample sizes (noted as non-estimable in Table 6).

DISCUSSION

We present results from the largest population-based study, to our knowledge, associating *MC1R*, phenotypes, and tumor characteristics with melanoma *BRAF/NRAS* subtypes. We confirm that *BRAF*+ and *NRAS*+ cases tend to be younger and older, respectively, than WT, as reported in GEM (Thomas et al., 2015, Thomas et al., 2007) and other studies (Hacker et al., 2010, Hacker et al., 2016, Liu et al., 2007, Viros et al., 2008). We also confirm prior reports that *BRAF* V600K tend to be older than V600E cases (Bucheit et al., 2013, Jewell et al., 2012, Menzies et al., 2012). We confirm in this larger GEM population our previous finding for North Carolina participants that increased nevi are associated with *BRAF*+ and approach significance for *NRAS*+ melanomas (Thomas et al., 2007). Hacker et al. similarly found an association of *BRAF* V600 tumors with increased nevi in an Australian population (Hacker et al., 2010). Comparing *BRAF* subgroups, we report here that *BRAF* V600E and V600K patients each have more nevi than WT, similar to the findings of Hacker et al. (Hacker et al., 2016). Our data and that of Hacker et al. (Hacker et al., 2016) indicate that *BRAF* V600E, V600K, and *NRAS*+ subtypes have nevogenic pathways. To our knowledge, no prior study has reported that *BRAF*+ relative to WT (or *NRAS*+) and *BRAF* V600E relative to WT are associated with blond/light brown hair or that *BRAF* V600K relative to WT (or V600E) is associated with less freckling. We previously found no association of

MC1R variants with *BRAF*+ melanomas compared with melanomas lacking *BRAF* mutations in the North Carolina GEM population (Thomas et al., 2010b). However, in the larger GEM population reported here, *MC1R* variants were positively associated with *BRAF* V600E compared with WT, but only among individuals with less sensitive phototypes or darker hair or eyes, and inversely associated with *BRAF* V600K. These results indicate distinct risk profiles for *BRAF, NRAS* and *WT* and also for *BRAF* V600E and V600K subtypes.

The results are also consistent with our prior report on first primary melanomas in GEM for tumor characteristics (Thomas et al., 2015, Thomas et al., 2007). We report here that, compared to WT, *BRAF*+ tumors were more likely to be SSM and truncal and have neval remnants but absent solar elastosis, and *NRAS*+ were more likely to be SSM or NM and thicker and arise on sites other than the head/neck, consistent with other studies (Bauer et al., 2011, Devitt et al., 2011, Edlundh-Rose et al., 2006, Ellerhorst et al., 2011, Hacker et al., 2010, Liu et al., 2007, Maldonado et al., 2003, Poynter et al., 2006). Our finding that *NRAS*+ compared with WT melanomas were less likely to have solar elastosis has not previously been reported to our knowledge. The higher likelihood of solar elastosis for WT compared with *BRAF* V600E or *NRAS*+ tumors indicates that cumulative sun exposure, the UV measure we found strongly associates with solar elastosis independent of age (Thomas et al., 2010c), may contribute more to WT than to *BRAF* V600E or *NRAS* tumor etiology. When compared with *BRAF* V600E melanomas, V600K tumors were positively associated both here and in the literature with head/neck locations (Bucheit et al., 2013) and adjacent solar elastosis (Menzies et al., 2012), indicating cumulative sun exposure may contribute to V600K melanomagenesis. Similar frequency of solar elastosis for V600K and WT tumors indicates cumulative sun exposure may contribute equally to these subtypes. The *BRAF* V600K mutation is a double base-pair substitution, and it has been hypothesized that the V600 tandem mutation may arise as a result of DNA repair of dipyrimidine dimers that form in response to ultraviolet damage (Thomas et al., 2004, Thomas et al., 2006).

We report here opposite associations of *BRAF* subtypes with *MC1R* risk variants: positive for V600E and inverse for V600K compared to WT melanoma. Notably, the associations of *MC1R* with V600E were strengthened and the associations of *MC1R* with V600K did not materially change when adjusted for phenotypes, indicating *MC1R* affects risk of the V600 subtypes independently of phenotype. *MC1R* could contribute to risk independent of fair phenotype if *MC1R* signaling activates antioxidant, DNA repair, survival, or immune pathways (Cao et al., 2013, Maresca et al., 2015, Nasti and Timares, 2015).

Opposing associations of *MC1R* with *BRAF* subtypes might explain, at least in part, prior reports of associations of *MC1R* positively with *BRAF*+ tumors arising on non-chronic sun-induced damaged (non-CSD) skin with low solar elastosis (Landi et al., 2006) and inversely with head/neck *BRAF*+ melanomas (Hacker et al., 2013). *BRAF* V600E tend not to have solar elastosis, while V600K tend to have it, as reported here and previously (Bucheit et al., 2013, Menzies et al., 2012). Restriction to non-CSD melanoma or head/neck site could enrich for either *BRAF* V600E or V600K melanomas, respectively. However, as similarly reported for the GEM North Carolina participants (Thomas et al., 2010b), we found no

statistically significant interactions with solar elastosis or sun-exposed site on the relationship between *MC1R* and *BRAF*+ melanoma in GEM.

The observed inconsistency in the association of *MC1R* with *BRAF*+ melanoma across studies in different countries (Fargnoli et al., 2008, Hacker et al., 2010, Hacker et al., 2016, Landi et al., 2006, Thomas et al., 2010b), including a lack of association in the GEM North Carolina population, might more completely be explained by our current findings in this larger study. Here, *MC1R* variants were associated with V600E compared with WT tumors only in individuals with increased tannability, decreased tendency to burn, darker hair, or darker eyes. Other studies have found overall melanoma risk conferred by *MC1R* genotypes was often stronger among persons with darker phenotypes (Dwyer et al., 2004, Kanetsky et al., 2010, Palmer et al., 2000, Pasquali et al., 2015). The clinical significance of their findings is that *MC1R* genotypes provide information about melanoma risk in individuals who would not be identified as high risk based on their phenotypes (Kanetsky et al., 2010). We extend this understanding as our study indicates that *MC1R* genotypes specifically provide information about *BRAF* V600E melanoma risk in those not considered high risk based on phenotype.

Individuals with dark phenotypes who carry high risk *MC1R* variants often do so in a heterozygous state, which likely accounts for the discordance between their inherited genetic risk and expressed phenotype. It also may be that their darker phenotype is due, in part, to the influence of other pigmentation genes. Our finding that *MC1R* was only associated with *BRAF* V600E melanoma in patients with darker phenotypes supports the findings by Mitra et al. (Mitra et al., 2012) that pigmentary genes may interact in relationship to *BRAF* V600E melanoma risk. Mitra et al. observed a high incidence of invasive melanomas independent of UV exposure in mice with a conditional, melanocyte-targeted allele of *BRAF* V600E and inactivating mutation in *MC1R* (with an analogous phenotype to red hair/fair skin in humans); however, tyrosinase deletion (with presumed loss of both eumelanin and pheomelanin production) abrogated melanoma susceptibility related to *MC1R* inactivation in this model. A report found the eumelanin to pheomelanin ratio in human epidermis is rather constant regardless of pigmentation, with a higher content of not only eumelanin but also pheomelanin with darker constitutive pigmentation (Del Bino et al., 2015). Yet, to our knowledge, pheomelanin levels have not been chemically measured in human epidermis and compared between the presence and absence of *MC1R* variants for darker and lighter phenotypes. Future work seems necessary to understand at a biologic level the findings of an interaction of *MC1R* with phenotypes for *BRAF* V600E melanoma. A carcinogenic effect of pheomelanin for *BRAF* V600E melanomagenesis could be mediated by metal ions, melanosomal amyloid, reactive oxygen species, or reactive nitrogen species generated by pheomelanin (Liu-Smith et al., 2015, Meyskens et al., 2004).

Our study's strengths are its population-based design, large sample size, and rigorous analysis of *BRAF* and *NRAS* mutations. Tumor tissue was obtained from 58% of index melanoma cases in four GEM study centers and analyzed for *BRAF* and *NRAS* mutations; and we found no significant differences comparing the distributions of sex, age, phenotypes or *MC1R* genotypes in cases with and without tumor mutation analysis (data not shown). The basis of tumors selected for *BRAF*/*NRAS* analysis was essentially arbitrary: from four

of nine GEM study centers in which it was judged feasible to obtain participants' formalin fixed tumor tissues. Study limitations include the lower statistical power for *BRAF* subgroup analyses, especially when examining interactions. Also, multiple tests were performed, which could lead to false discovery.

In conclusion, we find *BRAF*+ and *NRAS*+ melanomas have distinct profiles in relationship to age, phenotypes, and tumor characteristics. Further, *BRAF* V600K and V600E cases differed in age, freckling, *MC1R* variant carriage, tumor site, and solar elastosis, supporting the hypothesis that the risk profiles of these two subtypes are also genuinely distinct. Effect modification of phenotypes on *MC1R*'s association with *BRAF* V600E melanoma might explain inconsistencies in the literature regarding *MC1R*'s association with *BRAF*+ melanoma. Our result that *MC1R* was associated with *BRAF* V600E tumors only among those with darker phototypes indicates *MC1R* genotypes may provide information about *BRAF* V600E melanoma risk in individuals not identified as high risk based on phenotype. This interaction also supports an intrinsic pheomelanin pathway as contributing to *BRAF* V600E melanomagenesis. Additional investigation of the melanin pathways for a role in *BRAF* V600E melanomagenesis seems warranted. Explorations of the different melanoma pathways, of which our data suggest there are at least four (associated with *BRAF* V600E, *BRAF* V600K, *NRAS*+ and WT) with a focus on melanin as a potential risk factor for *BRAF* V600E melanoma could inform future risk models, prevention efforts, and chemoprevention for this complex disease.

MATERIALS AND METHODS

Study Population

The GEM study's 3579 participants had first or higher order primary melanoma diagnosed between 1998 and 2003 in Australia, Canada, Italy, and the United States (Begg et al., 2004, Begg et al., 2006, Millikan et al., 2006, Orlow et al., 2007). Each participating site's institutional review board approved the study protocol. Study participants provided written informed consent. For 2116 participants from New South Wales (Australia), California, North Carolina, and Michigan, we sought tissue sections from their first or higher order incident primary invasive melanoma that brought them into the GEM study. Of these 2116 participants, 1227 (58%) had formalin-fixed, paraffin-embedded melanoma tissues obtained and analyzed for *BRAF* exon 15 (including codon 600) and *NRAS* exon 2 and 3 (including codons 61, 12, and 13) mutations using single-strand conformational polymorphism (SSCP) analysis and radiolabeled sequencing of SSCP-positive samples (Thomas et al., 2015, Thomas et al., 2007). We previously reported the associations of *BRAF/NRAS* mutational status with age, sex, tumor characteristics, and survival for the 912 GEM first primaries (Thomas et al., 2015) and with age, sex, and phenotype in 214 GEM first primaries from North Carolina (Thomas et al., 2007) and 88 from Michigan (Poynter et al., 2006).

Age, sex, and phenotypes were collected from phone interviews and self-administered questionnaires (Kricker et al., 2007, Thomas et al., 2007, Thomas et al., 2010c). Number of back nevi has been reported previously as predictive of total body nevus counts (Autier et al., 2001, English and Armstrong, 1994, English et al., 1988). Diagnostic slides were

reviewed centrally for histopathologic criteria (Thomas et al., 2013, Thomas et al., 2015, Thomas et al., 2010c, Thomas et al., 2014).

MC1R was sequenced from buccal DNA (Kanetsky et al., 2006). *MC1R* genotypes were available for 1044 (85.1%) of the 1227 patients included here. Participants were classified as heterozygous, homozygous, or compound heterozygous carriers of higher [R] or lower [r] risk *MC1R* variants or *MC1R* consensus [wt], where [R] variants (D84E, R142H, R151C, R160W, D294H, and all nonsense and insertion/deletions) had a demonstrated strong association with red hair phenotype and [r] variants had a weaker association with red hair phenotype (Taylor et al., 2015).

Statistical Analyses

First, the melanomas were grouped as *BRAF*+ (exon 15 mutation), *NRAS*+ (exon 2 or 3 mutation), or WT. We used separate logistic regression models to estimate adjusted odds ratios (OR) and 95% confidence intervals (CI) comparing *BRAF*+ to WT, *NRAS*+ to WT, and *BRAF*+ to *NRAS*+ melanoma. We then conducted logistic regression analyses comparing *BRAF* V600E to WT, V600K to WT, and V600K to V600E melanoma. Further, in stratified analyses, we assessed effect modification by phenotypes of the association of *MC1R* with *BRAF* V600E and, separately, V600K (each compared with WT) and of V600K compared with V600E. All analyses included the covariates age, sex, and the study design variables: study center and status as first or higher order primary. Some analyses were also adjusted for phenotypes. Statistical significance was assessed using Wald tests; linear trend was assessed across ordinal categories of age and phenotypes. The likelihood ratio test was used to test interactions, comparing models with main effects to models with main effects and interaction terms. All statistical tests were two-sided with $P < 0.05$ considered statistically significant. All data were analyzed using SAS 9.3 (Cary, NC).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GEM	Genes, Environment, and Melanoma
IQR	interquartile range
WT	wildtype (without *BRAF* exon 15 or *NRAS* exon 2 and 3 mutations)

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Table 1

Characteristics of 1227 incident primary invasive cutaneous melanomas analyzed for *BRAF* and *NRAS* mutations in the GEM study

Characteristic	No. (%)
Sex	
Male	729 (59.4)
Female	498 (40.6)
Age at diagnosis, years	
Median (IQR)	60.0 (24.0)
< 50	343 (28.0)
50–69	505 (41.2)
> 70	379 (30.9)
Country	
Australia (New South Wales)	753 (61.4)
United States (NC, MI, and CA)	474 (38.6)
Lesion status	
First primary melanoma	912 (74.3)
Second or higher order primary melanoma	315 (25.7)
BRAF and *NRAS* mutation	
WT *(BRAF−/NRAS−)*	739 (60.2)
BRAF+	323 (26.3)
BRAF V600E	220 (17.9)[3]
BRAF V600K	68 (5.5)[3]
BRAF other[1]	35 (2.9)[3]
NRAS+	165 (13.5)
Histologic subtype	
Superficial Spreading	833 (67.9)
Nodular	104 (8.5)
Lentigo maligna	185 (15.1)
Unclassified/other[2]	105 (8.6)
Breslow thickness, mm	
Median (IQR), mm	0.70 (0.83)
0.01 to 1.00	817 (66.6)
1.01 to 2.00	250 (20.4)
2.01 to 4.00	113 (9.2)
>4.00	47 (3.8)
Anatomic site	
Trunk/pelvis	548 (44.7)
Head/neck	228 (18.6)
Upper extremities	228 (18.6)
Lower extremities	223 (18.2)

Abbreviations: GEM, Genes, Environment, and Melanoma; IQR, interquartile range; SD, standard deviation; WT, wildtype (without *BRAF* exon 15 or *NRAS* exon 2 and 3 mutations).

[1] *BRAF* other included: L584F (n = 1), D594G (n = 2), D594N (n = 6), L597K (n = 1), L597R (n = 1), L597S (n = 1), V600D (n = 4), V600R (n = 9), K601E (n = 5), K601N (n = 2), R603Q (n = 1), G606E (n = 1), and the compound deletion VKS600-602D (n = 1).

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

[3] Percent refers to of all melanomas.

Table 2

Tumor characteristics: ORs and 95% CIs associated with *BRAF*+ and *NRAS*+ melanomas in 1227 patients

	WT (n = 739)	*BRAF*+ (n = 323)	*NRAS*+ (n = 165)	*BRAF*+ vs. WT		*NRAS*+ vs. WT		*BRAF*+ vs. *NRAS*+	
Tumor characteristic	**n (%)[1]**	**n (%)[1]**	**n (%)[1]**	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***
Histologic subtype									
Superficial Spreading	465 (62.9)	267 (82.7)	101 (61.2)	1.0 (referent)	<.001	1.0 (referent)	<.001	1.0 (referent)	<.001
Nodular	46 (6.2)	22 (6.8)	36 (21.8)	0.95 (0.55–1.64)		3.42 (2.07–5.65)		0.32 (0.17–0.58)	
Lentigo maligna	150 (20.3)	24 (7.4)	11 (6.7)	0.37 (0.23–0.59)		0.29 (0.15–0.56)		1.31 (0.59–2.91)	
Unclassified/other	78 (11.6)	10 (3.1)	17 (10.3)	0.25 (0.13–0.51)		0.90 (0.50–1.61)		0.31 (0.13–0.73)	
Breslow thickness, mm									
Median (IQR), mm	0.62 (0.70)	0.80 (0.78)	1.13 (1.36)						
0.01–1.00	533 (72.1)	207 (64.1)	77 (46.7)	1.0 (referent)	.01	1.0 (referent)	<.001	1.0 (referent)	.002
1.01–2.00	122 (16.5)	83 (25.7)	45 (27.3)	1.73 (1.23–2.42)		2.49 (1.64–3.81)		0.70 (0.43–1.12)	
2.01–4.00	59 (8.0)	25 (7.7)	29 (17.6)	1.15 (0.69–1.93)		3.00 (1.79–5.03)		0.40 (0.22–0.76)	
>4.00	25 (3.4)	8 (2.5)	14 (8.5)	0.85 (0.37–1.97)		3.82 (1.88–7.77)		0.25 (0.10–0.65)	
Anatomic site									
Trunk/pelvis	304 (41.1)	173 (53.6)	71 (43.0)	1.0 (referent)	.003	1.0 (referent)	<.001	1.0 (referent)	.05
Head/neck	171 (23.1)	40 (12.4)	17 (10.3)	0.49 (0.33–0.74)		0.37 (0.21–0.66)		1.22 (0.63–2.38)	
Upper extremities	131 (17.7)	51 (15.8)	46 (27.9)	0.65 (0.44–0.96)		1.36 (0.87–2.12)		0.50 (0.30–0.86)	
Lower extremities	133 (18.0)	59 (18.3)	31 (18.8)	0.70 (0.47–1.03)		0.89 (0.54–1.49)		0.84 (0.47–1.48)	
Neval remnants									
Absent	559 (75.6)	213 (65.9)	120 (72.7)	1.0 (referent)	<.001	1.0 (referent)	.26	1.0 (referent)	.11
Present	180 (24.4)	110 (34.1)	45 (27.3)	1.77 (1.31–2.41)		1.26 (0.84–1.87)		1.45 (0.92–2.26)	
Solar elastosis									
Absent	146 (20.1)	100 (31.6)	41 (25.5)	1.0 (referent)	.003	1.0 (referent)	.03	1.0 (referent)	.82
Present	579 (79.9)	217 (68.5)	120 (74.5)	0.61 (0.44–0.84)		0.63 (0.41–0.96)		0.95 (0.59–1.52)	

Abbreviations: IQR, interquartile range; WT, wildtype (without *BRAF* exon 15 or *NRAS* exon 2 and 3 mutations).

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

[2] Covariates include age (<50, 50–69, >70), sex, study center, and status as first or higher order primary.

Table 3

Age, sex, phenotypes and *MC1R* genotype: ORs and 95% CIs with *BRAF*+ and *NRAS*+ melanomas in 1227 patients

	WT (n = 739)	*BRAF*+ (n = 323)	*NRAS*+ (n = 165)	*BRAF*+ vs. WT		*NRAS*+ vs. WT		*BRAF*+ vs. *NRAS*+	
Characteristic	**n (%)[1]**	**n (%)[1]**	**n (%)[1]**	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***
Sex									
Male	455 (61.6)	180 (55.7)	94 (57.0)	1.0 (referent)	.89	1.0 (referent)	.11	1.0 (referent)	.20
Female	284 (38.4)	143 (44.3)	71 (43.0)	1.02 (0.77–1.36)		1.33 (0.93–1.90)		0.80 (0.53–1.21)	
Age at diagnosis, years									
Median (IQR)	63.0 (23.0)	53.0 (24.0)	65.0 (20.0)						
< 50	179 (24.2)	137 (42.4)	27 (16.4)	1.0 (referent)	<.001	1.0 (referent)	.03	1.0 (referent)	<.001
50–69	307 (41.5)	123 (38.1)	75 (45.5)	0.61 (0.44–0.85)	P_{trend}	1.84 (1.12–3.01)	P_{trend}	0.33 (0.19–0.55)	P_{trend}
> 70	253 (34.2)	63 (19.5)	63 (38.2)	0.41 (0.28–0.59)		1.91 (1.14–3.18)		0.21 (0.12–0.38)	
Tannability									
Deep tan	108 (14.9)	58 (18.2)	24 (14.8)	1.0 (referent)	.22	1.0 (referent)	.83	1.0 (referent)	.14
Moderate tan	312 (43.1)	134 (42.1)	62 (38.3)	0.81 (0.55–1.21)	P_{trend}	0.91 (0.54–1.54)	P_{trend}	0.90 (0.50–1.62)	P_{trend}
Mild tan	205 (28.3)	96 (30.2)	54 (33.3)	0.90 (0.59–1.37)		1.15 (0.67–1.99)		0.75 (0.40–1.39)	
No tan	99 (13.7)	30 (9.4)	22 (13.6)	0.62 (0.36–1.07)		0.90 (0.47–1.74)		0.60 (0.27–1.30)	
Tendency to burn									
No burn	51 (7.1)	29 (9.1)	10 (6.2)	1.0 (referent)	.83	1.0 (referent)	.91	1.0 (referent)	.55
Burn then tan	347 (48.0)	136 (42.6)	79 (48.8)	0.60 (0.36–1.01)	P_{trend}	1.17 (0.56–2.42)	P_{trend}	0.52 (0.23–1.17)	P_{trend}
Burn then peel	257 (35.6)	133 (41.7)	57 (35.2)	0.81 (0.48–1.38)		1.16 (0.55–2.46)		0.69 (0.30–1.57)	
Burn with blister	68 (9.4)	21 (6.6)	16 (9.9)	0.55 (0.27–1.10)		1.12 (0.46–2.70)		0.41 (0.15–1.15)	
Hair color									
Dark brown/black	206 (28.0)	76 (23.5)	56 (33.9)	1.0 (referent)	.05	1.0 (referent)	.34	1.0 (referent)	.03
Blonde/light brown	456 (62.0)	222 (68.7)	97 (58.8)	1.37 (0.99–1.89)		0.82 (0.56–1.19)		1.83 (1.17–2.87)	
Red	74 (10.1)	25 (7.7)	12 (7.3)	0.85 (0.49–1.47)		0.63 (0.32–1.24)		1.44 (0.64–3.27)	
Eye color									
Brown	137 (18.6)	63 (19.6)	27 (16.4)	1.0 (referent)	.29	1.0 (referent)	.22	1.0 (referent)	.73
Green/grey/hazel	298 (40.4)	117 (36.3)	62 (37.6)	0.94 (0.64–1.38)	P_{trend}	1.04 (0.63–1.72)	P_{trend}	0.95 (0.53–1.71)	P_{trend}
Blue	302 (41.0)	142 (44.1)	76 (46.1)	1.16 (0.80–1.70)		1.29 (0.79–2.11)		0.91 (0.52–1.60)	

Characteristic	WT (n = 739) n (%)[1]	*BRAF*+ (n = 323) n (%)[1]	*NRAS*+ (n = 165) n (%)[1]	*BRAF*+ vs. WT OR (95%CI)[2]	*P*	*NRAS*+ vs. WT OR (95%CI)[2]	*P*	*BRAF*+ vs. *NRAS*+ OR (95%CI)[2]	*P*
Number of back nevi									
0–4	285 (39.2)	89 (28.0)	57 (35.2)	1.0 (referent)	.02	1.0 (referent)	.08	1.0 (referent)	.89
5–10	169 (23.3)	86 (27.0)	42 (25.9)	1.58 (1.10–2.29)	P_{trend}	1.39 (0.88–1.18)	P_{trend}	1.23 (0.73–2.06)	P_{trend}
11–25	162 (22.3)	64 (20.1)	35 (21.6)	1.06 (0.73–1.60)		1.23 (0.76–1.98)		0.84 (0.47–1.48)	
>25	111 (15.3)	79 (24.8)	28 (17.3)	1.91 (1.28–2.87)		1.69 (0.99–2.89)		1.17 (0.65–2.12)	
Freckles in childhood									
None	294 (41.6)	146 (47.4)	68 (41.7)	1.0 (referent)	.03	1.0 (referent)	.92	1.0 (referent)	.09
Few	319 (45.1)	128 (41.6)	75 (46.0)	0.78 (0.58–1.05)	P_{trend}	1.04 (0.72–1.51)	P_{trend}	0.72 (0.46–1.10)	P_{trend}
Many	94 (13.3)	34 (11.0)	20 (12.3)	0.64 (0.40–1.02)		1.00 (0.57–1.76)		0.63 (0.32–1.22)	
MC1R[3]	(n = 616)	(n = 288)	(n = 140)						
wt/wt	90 (14.6)	44 (15.3)	19 (13.6)	1.0 (referent)	.78	1.0 (referent)	.93	1.0 (referent)	.94
r/wt or r/r	197 (32.0)	83 (28.8)	47 (33.6)	0.78 (0.49–1.24)	P_{trend}	1.10 (0.61–2.00)	P_{trend}	0.68 (0.34–1.36)	P_{trend}
R/R, R/r, or R/wt	329 (53.4)	161 (55.9)	74 (52.9)	0.96 (0.62–1.47)		1.06 (0.60–1.86)		0.88 (0.46–1.67)	
wt/wt	90 (14.6)	44 (15.3)	19 (13.6)	1.0 (referent)	.58	1.0 (referent)	.79	1.0 (referent)	.49
Any r or R	526 (85.4)	244 (84.7)	121 (86.4)	0.89 (0.59–1.34)		1.08 (0.63–1.85)		0.80 (0.43–1.49)	
wt/wt, r/wt or r/r	287 (46.6)	127 (44.1)	66 (47.1)	1.0 (referent)	.42	1.0 (referent)	.95	1.0 (referent)	.55
R/R, R/r, or R/wt	329 (53.4)	161 (55.9)	74 (52.9)	1.13 (0.84–1.52)		0.98 (0.68–1.44)		1.14 (0.74–1.75)	

Abbreviations: IQR, interquartile range; WT, wildtype (without *BRAF* exon 15 or *NRAS* exon 2 and 3 mutations). *MC1R*, "R": D84E, R142H, R151C, R160W, and D294H, all nonsense and insertion/deletion; "r": all other nonsynoymous variants; "wt": consensus. Compound heterozygotes for MC1R were classified as R/R if both mutations were higher risk for melanoma (either D84E, R142H, R151C, R160W, D294H, or nonsense and insertion/deletions); R/r if one mutation was higher risk for melanoma and the other was lower risk for melanoma based on weaker association with red hair phenotype); or r/r if both mutations were lower risk for melanoma.

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

[2] Covariates include age (<50, 50–69, >70), sex, study center, and status as first or higher order primary.

[3] 1044 patients had germline *MC1R* sequencing results.

Table 4

Tumor characteristics: ORs and 95% CIs associated with *BRAF* V600K and V600E vs. WT and V600K vs. V600E mutational status in 1027 patients

	WT (n = 739)	*BRAF* V600E (n = 220)	*BRAF* V600K (n = 68)	*BRAF* V600E vs. WT		*BRAF* V600K vs. WT		*BRAF* V600K vs. V600E	
Tumor characteristic	**n (%)[1]**	**n (%)[1]**	**n (%)[1]**	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***
Histologic subtype									
Superficial Spreading	465 (62.9)	191 (86.8)	56 (82.4)	1.0 (referent)	<.001	1.0 (referent)	.02	1.0 (referent)	.22
Nodular	46 (6.2)	15 (6.8)	4 (5.9)	1.00 (0.53–1.88)		0.65 (0.22–1.91)		0.56 (0.17–1.87)	
Lentigo maligna	150 (20.3)	7 (3.2)	7 (10.3)	0.17 (0.08–0.38)		0.38 (0.17–0.88)		2.75 (0.83–9.08)	
Unclassified/other	78 (11.6)	7 (3.2)	1 (1.5)	0.27 (0.11–0.60)		0.10 (0.01–0.73)		0.48 (0.05–4.27)	
Breslow thickness, mm									
Median (IQR), mm	0.62 (0.70)	0.85 (0.71)	0.69 (0.70)						
0.01–1.00	533 (72.1)	135 (61.4)	49 (72.1)	1.0 (referent)	.002	1.0 (referent)	.98	1.0 (referent)	.24
1.01–2.00	122 (16.5)	60 (27.3)	12 (17.7)	1.99 (1.34–2.95)		1.03 (0.53–2.03)		0.53 (0.25–1.12)	
2.01–4.00	59 (8.0)	19 (8.6)	5 (7.4)	1.45 (0.80–2.62)		0.85 (0.32–2.27)		0.44 (0.14–1.36)	
>4.00	25 (3.4)	6 (2.7)	2 (2.9)	1.02 (0.39–2.68)		0.84 (0.19–3.73)		0.67 (0.12–3.78)	
Anatomic site									
Trunk/pelvis	304 (41.1)	120 (54.6)	34 (50.0)	1.0 (referent)	<.001	1.0 (referent)	.43	1.0 (referent)	.005
Head/neck	171 (23.1)	17 (7.7)	15 (22.1)	0.30 (0.17–0.53)		0.84 (0.44–1.63)		3.68 (1.55–8.74)	
Upper extremities	131 (17.7)	35 (15.9)	12 (17.7)	0.61 (0.38–0.96)		0.82 (0.40–1.68)		1.45 (0.63–3.33)	
Lower extremities	133 (18.0)	48 (21.8)	7 (10.3)	0.74 (0.47–1.15)		0.47 (0.19–1.14)		0.52 (0.20–1.33)	
Neval remnants									
Absent	559 (75.6)	140 (63.6)	48 (70.6)	1.0 (referent)	<.001	1.0 (referent)	.22	1.0 (referent)	.05
Present	180 (24.4)	80 (36.4)	20 (29.4)	2.11 (1.47–3.01)		1.43 (0.80–2.56)		0.51 (0.26–0.98)	
Solar elastosis									
Absent	146 (20.1)	84 (39.1)	11 (16.4)	1.0 (referent)	<.001	1.0 (referent)	.40	1.0 (referent)	.001
Present	579 (79.9)	131 (60.9)	56 (83.6)	0.44 (0.30–0.63)		1.36 (0.67–2.73)		3.45 (1.61–7.36)	

Abbreviations: IQR, interquartile range; WT, wildtype (without *BRAF* exon 15 or *NRAS* exon 2 and 3 mutations).

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

[2] Covariates include age (<50, 50–69, >70), sex, study center, and status as first or higher order primary.

Table 5

Age, sex, and phenotypes and *MC1R* genotype: ORs and 95% CIs associated with *BRAF* V600K and V600E vs. WT and V600K vs. V600E mutational status in 1027 patients

	WT (n = 739)	*BRAF* V600E (n = 220)	*BRAF* V600K (n = 68)	*BRAF* V600E vs. WT		*BRAF* V600K vs. WT		*BRAF* V600K vs. V600E	
Characteristic	**n (%)[1]**	**n (%)[1]**	**n (%)[1]**	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***
Sex									
Male	455 (61.6)	100 (50.0)	46 (67.7)	1.0 (referent)	.34	1.0 (referent)	.18	1.0 (referent)	.08
Female	284 (38.4)	110 (50.0)	22 (32.4)	1.18 (0.84–1.64)		0.69 (0.40–1.19)		0.59 (0.32–1.07)	
Age at diagnosis, years									
Median (IQR)	63.0 (23.0)	49.0 (25.5)	56.0 (24.0)						
< 50	179 (24.2)	113 (51.4)	18 (26.5)	1.0 (referent)	<.001	1.0 (referent)	.63	1.0 (referent)	.003
50–69	307 (41.5)	76 (34.6)	30 (44.1)	0.51 (0.35–0.73)	P_{trend}	1.04 (0.55–1.97)	P_{trend}	2.09 (1.07–4.09)	P_{trend}
> 70	253 (34.2)	31 (14.1)	20 (29.4)	0.27 (0.17–0.42)		0.86 (0.42–1.72)		3.27 (1.47–7.27)	
Tannability									
Deep tan	108 (14.9)	44 (20.3)	8 (11.9)	1.0 (referent)	.32	1.0 (referent)	.65	1.0 (referent)	0.36
Moderate tan	312 (43.1)	83 (38.3)	37 (55.2)	0.64 (0.41–1.01)	P_{trend}	1.67 (0.75–3.74)	P_{trend}	3.19 (1.31–7.75)	P_{trend}
Mild tan	205 (28.3)	73 (33.6)	14 (20.9)	0.91 (0.57–1.48)		0.99 (0.40–2.48)		1.35 (0.50–3.66)	
No tan	99 (13.7)	17 (7.8)	8 (11.9)	0.50 (0.25–0.97)		1.20 (0.42–3.40)		3.96 (1.15–13.63)	
Tendency to burn									
No burn	51 (7.1)	20 (9.2)	6 (9.0)	1.0 (referent)	.63	1.0 (referent)	.70	1.0 (referent)	.18
Burn then tan	347 (48.0)	92 (42.4)	29 (43.3)	0.54 (0.29–0.98)	P_{trend}	0.67 (0.26–1.72)	P_{trend}	1.58 (0.53–4.69)	P_{trend}
Burn then peel	257 (35.6)	94 (43.3)	25 (37.3)	0.74 (0.40–1.38)		0.84 (0.32–2.18)		1.38 (0.46–4.15)	
Burn with blister	68 (9.4)	11 (5.1)	7 (10.5)	0.41 (0.17–0.98)		0.91 (0.28–2.92)		4.74 (1.11–20.18)	
Hair color									
Dark brown/black	206 (28.0)	44 (20.0)	21 (30.9)	1.0 (referent)	.03	1.0 (referent)	.56	1.0 (referent)	.16
Blonde/light brown	456 (62.0)	156 (70.9)	43 (63.2)	1.67 (1.12–2.48)		0.97 (0.56–1.69)		0.53 (0.27–1.02)	
Red	74 (10.1)	20 (9.1)	4 (5.9)	1.15 (0.61–2.14)		0.55 (0.18–1.69)		0.52 (0.15–1.80)	
Eye color									
Brown	137 (18.6)	39 (17.8)	13 (19.1)	1.0 (referent)	.19	1.0 (referent)	.49	1.0 (referent)	.73
Green/grey/hazel	298 (40.4)	84 (38.4)	23 (33.8)	1.14 (0.72–1.81)	P_{trend}	0.86 (0.42–1.76)	P_{trend}	0.65 (0.28–1.49)	P_{trend}

Characteristic	WT (n = 739) n (%)[1]	*BRAF* V600E (n = 220) n (%)[1]	*BRAF* V600K (n = 68) n (%)[1]	*BRAF* V600E vs. WT OR (95%CI)[2]	*P*	*BRAF* V600K vs. WT OR (95%CI)[2]	*P*	*BRAF* V600K vs. V600E OR (95%CI)[2]	*P*
Blue	302 (41.0)	96 (43.8)	32 (47.1)	1.34 (0.85–2.11)		1.17 (0.59–2.34)		0.78 (0.35–1.73)	
Number of back nevi									
0–4	285 (39.2)	54 (24.8)	20 (30.3)	1.0 (referent)	.04	1.0 (referent)	.01	1.0 (referent)	.17
5–10	169 (23.3)	67 (30.7)	12 (18.2)	2.04 (1.33–3.14)	P_{trend}	1.07 (0.48–2.18)	P_{trend}	0.48 (0.20–1.10)	P_{trend}
11–25	162 (22.3)	42 (19.3)	16 (24.2)	1.08 (0.67–1.75)		1.46 (0.72–2.95)		1.55 (0.67–3.63)	
>25	111 (15.3)	55 (25.2)	18 (27.3)	2.05 (1.27–3.32)		2.60 (1.26–5.35)		1.39 (0.61–3.16)	
Freckles in childhood									
None	294 (41.6)	92 (44.4)	43 (65.2)	1.0 (referent)	.18	1.0 (referent)	<.001	1.0 (referent)	.02
Few	319 (45.1)	89 (43.0)	20 (30.3)	0.81 (0.57–1.16)	P_{trend}	0.45 (0.25–0.79)	P_{trend}	0.53 (0.29–1.02)	P_{trend}
Many	94 (13.3)	26 (12.6)	3 (4.6)	0.74 (0.43–1.26)		0.23 (0.07–0.77)		0.32 (0.09–1.15)	
MC1R[3]	(n = 616)	(n = 201)	(n = 54)						
wt/wt	90 (14.6)	22 (11.0)	18 (33.3)	1.0 (referent)	.08	1.0 (referent)	.006	1.0 (referent)	.002
r/wt or r/r	197 (32.0)	59 (29.4)	13 (24.1)	1.13 (0.63–2.02)	P_{trend}	0.31 (0.14–0.67)	P_{trend}	0.27 (0.10–0.68)	P_{trend}
R/R, R/r, or R/wt	329 (53.4)	120 (59.7)	23 (42.6)	1.48 (0.86–2.56)		0.34 (0.17–0.66)		0.24 (0.11–0.55)	
wt/wt	90 (14.6)	22 (11.0)	18 (33.3)	1.0 (referent)	.33	1.0 (referent)	<.001	1.0 (referent)	<.001
Any r or R	526 (85.4)	179 (89.1)	36 (66.7)	1.34 (0.79–2.23)		0.32 (0.18–0.61)		0.25 (0.12–0.54)	
wt/wt, r/wt or r/r	287 (46.6)	81 (40.3)	31 (57.4)	1.0 (referent)	.07	1.0 (referent)	.14	1.0 (referent)	.04
R/R, R/r, or R/wt	329 (53.4)	120 (69.7)	23 (42.6)	1.36 (0.96–1.93)		0.65 (0.37–1.16)		0.51 (0.33–0.97)	

Abbreviations: IQR, interquartile range; WT, wildtype (without *BRAF* exon 15 or *NRAS* exon 2 and 3 mutations). *MC1R*, "R": D84E, R142H, R151C, R160W, and D294H, all nonsense and insertion/deletion; "r": all other variants; "wt": consensus.

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

[2] Covariates include age (<50, 50–69, >70), sex, study center, and status as first or higher order primary.

[3] 871 patients had germline *MC1R* sequencing results.

Table 6

MC1R genotype: ORs and 95% CIs for each of *BRAF* V600E and V600K vs. WT and V600K vs. V600E mutational subtype by phenotypes in 871 patients

	WT	*BRAF* V600E	*BRAF* V600K	*BRAF* V600E vs. WT		*BRAF* V600K vs. WT		*BRAF* V600K vs. V600E	
MC1R	**n (%)[1]**	**n (%)[1]**	**n (%)[1]**	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***
Stratification by phenotypes									
Deep/moderate tan	(n = 338)	(n = 118)	(n = 32)						
wt/wt	65 (19.2)	13 (11.0)	12 (37.5)	Reference	.004	Reference	.11	Reference	.008
r/wt or r/r	131(38.8)	37 (31.4)	8 (25.0)	1.47 (0.71–3.07)	P_{trend}	0.33 (0.13–0.86)	P_{trend}	0.21 (0.06–0.72)	P_{trend}
R/R, R/r, or R/wt	142 (42.0)	68 (57.6)	12 (37.5)	2.50 (1.24–5.04)		0.46 (0.19–1.10)		0.19 (0.06–0.55)	
Mild/no tan	(n = 264)	(n = 81)	(n = 21)						
wt/wt	21 (8.0)	9 (11.1)	6 (28.6)	Reference	.54	Reference	.02	Reference	.22
r/wt or r/r	63 (23.9)	21 (25.9)	4 (19.1)	0.52 (0.18–1.48)	P_{trend}	0.17 (0.04–0.70)	P_{trend}	0.30 (0.05–1.72)	P_{trend}
R/R, R/r, or R/wt	180 (68.2)	51 (63.0)	11 (52.4)	0.59 (0.23–1.54)		0.19 (0.06–0.61)		0.35 (0.09–1.42)	
$P_{interaction}$[3]					.01		.33		.54
No burn/burn then tan	(n = 326)	(n = 104)	(n = 27)						
wt/wt	65 (19.9)	8 (7.7)	13 (48.2)	Reference	.003	Reference	.006	Reference	.002
r/wt or r/r	116 (35.6)	37 (35.6)	6 (22.2)	2.53 (1.08–5.94)	P_{trend}	0.23 (0.08–0.66)	P_{trend}	0.09 (0.02–0.36)	P_{trend}
R/R, R/r, or R/wt	145 (44.5)	59 (56.7)	8 (29.6)	3.50 (1.53–8.01)		0.25 (0.10–0.66)		0.07 (0.02–0.24)	
Burn then peel or blister	(n = 274)	(n = 95)	(n = 26)						
wt/wt	22 (8.0)	14 (14.7)	5 (19.2)	Reference	.32	Reference	.22	Reference	.77
r/wt or r/r	76 (27.7)	21 (22.1)	6 (23.1)	0.35 (0.14–0.89)	P_{trend}	0.29 (0.08–1.12)	P_{trend}	1.17 (0.25–5.49)	P_{trend}
R/R, R/r, or R/wt	176 (64.2)	60 (63.2)	15 (57.7)	0.47 (0.20–1.08)		0.35 (0.11–1.12)		0.90 (0.25–3.23)	
$P_{interaction}$[3]					.005		.41		.01
Dark brown/black hair	(n =176)	(n = 42)	(n = 15)						
wt/wt	33 (18.8)	2 (4.8)	8 (53.3)	Reference	.01	*non–estimable*		*non–estimable*	
r/wt or r/r	69 (39.2)	17 (40.5)	3 (20.0)	5.08 (0.97–26.52)	P_{trend}				
R/R, R/r, or R/wt	74 (42.1)	23 (54.8)	4 (26.7)	7.90 (1.51–41.23)					
Blonde/light brown/red hair	(n = 437)	(n = 159)	(n = 39)						
wt/wt	55 (12.6)	20 (12.6)	10 (25.6)	Reference	.83	Reference	.22	Reference	.12

	WT	*BRAF* V600E	*BRAF* V600K	*BRAF* V600E vs. WT		*BRAF* V600K vs. WT		*BRAF* V600K vs. V600E	
MC1R	**n (%)[1]**	**n (%)[1]**	**n (%)[1]**	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***
r/wt or r/r	128 (29.3)	42 (26.4)	10 (25.6)	0.76 (0.39–1.47)	P_{trend}	0.42 (0.16–1.09)	P_{trend}	0.56 (0.18–1.69)	P_{trend}
R/R, R/r, or R/wt	254 (58.1)	97 (61.0)	19 (48.6)	0.93 (0.51–1.71)		0.40 (0.17–0.92)		0.46 (0.18–1.18)	
$P_{interaction}$[3]					.05				
Brown eyes	(n = 122)	(n = 36)	(n = 11)						
wt/wt	23 (18.9)	2 (5.6)	5 (45.5)	Reference	.02	*non–estimable*		*non–estimable*	
r/wt or r/r	41 (33.6)	12 (33.3)	2 (18.2)	3.73 (0.70–20.01)	P_{trend}				
R/R, R/r, or R/wt	58 (47.5)	22 (61.1)	4 (36.4)	6.59 (1.29–33.78)					
Green/grey//hazel/blue eyes	(n = 492)	(n = 164)	(n = 43)						
wt/wt	65 (13.2)	20 (12.2)	13 (30.2)	Reference	.55	Reference	.01	Reference	.03
r/wt or r/r	156 (31.7)	46 (28.1)	11 (25.6)	0.80 (0.42–1.52)	P_{trend}	0.33 (0.14–0.78)	P_{trend}	0.39 (0.14–1.10)	P_{trend}
R/R, R/r, or R/wt	271 (55.1)	98 (59.8)	19 (44.2)	1.04 (0.57–1.87)		0.33 (0.15–0.71)		0.34 (0.14–0.84)	
$P_{interaction}$[3]					.06				
Back nevi 0–10	(n = 376)	(n = 109)	(n = 24)						
wt/wt	55 (14.6)	13 (11.9)	6 (25.0)	Reference	.22	Reference	.32	Reference	.16
r/wt or r/r	115 (30.6)	32 (29.4)	6 (25.0)	1.18 (0.55–2.52)	P_{trend}	0.46 (0.14–1.52)	P_{trend}	0.45 (0.11–1.86)	P_{trend}
R/R, R/r, or R/wt	206 (54.8)	64 (58.7)	12 (50.0)	1.47 (0.37–2.97)		0.52 (0.18–1.48)		0.35 (0.10–1.20)	
Back nevi >10	(n = 229)	(n = 91)	(n = 29)						
wt/wt	33 (14.4)	9 (9.9)	12 (41.4)	Reference	.25	Reference	.003	Reference	.005
r/wt or r/r	81 (35.4)	27 (29.7)	7 (24.1)	0.95 (0.37–2.40)	P_{trend}	0.18 (0.06–0.54)	P_{trend}	0.20 (0.05–0.81)	
R/R, R/r, or R/wt	115 (50.2)	55 (60.4)	10 (34.5)	1.38 (0.57–3.32)		0.19 (0.07–0.50)		0.15 (0.04–0.53)	
$P_{interaction}$[3]					.83		.16		.41
No freckles	(n = 238)	(n = 82)	(n = 34)						
wt/wt	55 (23.1)	18 (22.0)	13 (38.2)	Reference	.58	Reference	.09	Reference	.13
r/wt or r/r	90 (37.8)	31 (37.8)	11 (32.4)	1.05 (0.52–2.15)	P_{trend}	0.50 (0.21–1.21)	P_{trend}	0.46 (0.15–1.40)	P_{trend}
R/R, R/r, or R/wt	93 (39.1)	33 (40.2)	10 (29.4)	1.21 (0.59–2.47)		0.46 (0.18–1.13)		0.40 (0.13–1.20)	
Few/many freckles	(n = 352)	(n = 107)	(n = 18)						
wt/wt	27 (7.7)	3 (2.8)	5 (27.8)	Reference	.02	*non–estimable*		*non–estimable*	
r/wt or r/r	97 (27.6)	22 (20.6)	1 (5.6)	1.65 (0.43–6.31)	P_{trend}				

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	WT	*BRAF* V600E	*BRAF* V600K	*BRAF* V600E vs. WT		*BRAF* V600K vs. WT		*BRAF* V600K vs. V600E	
MC1R	**n (%)[1]**	**n (%)[1]**	**n (%)[1]**	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***	**OR (95%CI)[2]**	***P***
R/R, R/r, or R/wt	228 (64.8)	82 (76.6)	12 (66.7)	2.76 (0.77–9.91)					
$P_{interaction}$[3]					.20				

Abbreviations: WT, wildtype (without *BRAF* exon 15 or *NRAS* exon 2 and 3 mutations). *MC1R,* "R": D84E, R142H, R151C, R160W, and D294H, all nonsense and insertion/deletion; "r": all other variants; "wt": consensus;

[1]Counts may not sum-the total number of study subjects due-missing data.

[2]Covariates include age (<50, 50–69, >70), sex, study center, and status as first or higher order primary.

[3]*P* for interaction was determined for the adjusted model with and without the interaction term using the likelihood ratio test.