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# *MITF* E318K's effect on melanoma risk independent of, but modified by, other risk factors

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## Summary

A rare germline variant in the *MITF* (microphthalmia-associated transcription factor) gene, E318K, has been reported as associated with melanoma. We confirmed its independent association with melanoma (odds ratio (OR) 1.7, 95% Confidence Interval (CI) = 1.1, 2.7, $p = 0.03$); adjusted for age, sex, center, age*sex interaction, pigmentation characteristics, family history of melanoma and nevus density). In stratified analyses, carriage of *MITF* E318K was associated with melanoma more strongly in people with dark hair than fair hair ($p$ for interaction, 0.03) and in those with no moles than some or many moles ($p$ for interaction, <0.01). There was no evidence of interaction between *MC1R* "red hair variants" and *MITF* E318K. Moreover, risk of melanoma among carriers with "low risk" phenotypes was as great or greater than among those with "at risk" phenotypes with few exceptions.

**Keywords**

*MITF*; melanoma; risk factors; single nucleotide polymorphism; case control study

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*MITF*, the microphthalmia-associated transcription factor, is critical for lineage commitment of undifferentiated, immature neural crest cells to mature, melanin-producing melanocytes (Goding, 2000). UV exposure to the epidermis promotes alpha-MSH release, which then binds to the melanocortin-1 receptor (*MC1R*), a transmembrane G-protein-coupled receptor located on the cell membrane of melanocytes. This binding culminates in the activation of the M-isoform of *MITF*, termed MITF-M, expressed in melanocytes through a cAMP-

mediated signaling pathway (Haq and Fisher, 2011; Fuse et al., 1996). The activation of *MITF*, in turn, induces the transcription of pigmentation-related genes, which produce eumelanin that protects cells from UV damage (Cheli et al., 2009). Other MITF functions may be based on the fact that several MITF-target genes regulate cell cycle and survival (Cheli et al., 2010), and MITF appears to protect against oxidative stress (Liu et al., 2009).

The E318K variant of *MITF* (rs149617956) is a functional and rare variant that is associated with melanoma risk. It is located in a small-ubiquitin-like modifier (SUMO) consensus site and the missense variant impairs the SUMOylation of *MITF*, leading to the binding of *MITF* and the transcriptional regulation of *MITF*'s target genes. In a large Australian case-control study of 2,059 melanoma cases and in a UK case-control study of 1,929 cases, the E318K variant conferred a 2.2-fold risk for developing melanoma (Yokoyama et al., 2011). Among a sample of 586 French melanoma patients, genetically enriched for melanoma and renal cell carcinoma, a 4.8-fold increased risk of melanoma was observed in *MITF* variant carriers (Bertolotto et al., 2011). An Italian study of 667 patients in a clinic setting found a 2.9-fold increased risk for melanoma, which was greater with nodular than with other histological types of melanoma (Ghiorzo et al., 2013). They also observed positive associations of the variant with personal or family history of renal cell or pancreatic cancer.

The prevalence of the *MITF* E318K minor allele variant among melanoma cases is low: 0.017 in Australia; 0.018 in the UK; 0.016 in France; and 0.009 in Italy (Yokoyama et al., 2011; Bertolotto et al., 2011; Ghiorzo et al., 2013). Our large international, multicenter melanoma case control study, GEM, was designed especially to identify the effects of rare genetic variants in melanoma (Begg and Berwick, 1997). A detailed description of the methods used in this study is available elsewhere (Begg et al., 2006). It was conducted in nine centers in four countries--Australia, Italy, Canada and the United States. Institutional review board approval was obtained from all centers. Written informed consent was obtained prior to interview.

In GEM, patients newly diagnosed with a second or higher order primary melanoma are cases, and individuals newly diagnosed with a first primary melanoma are controls; this design produces similar results to classic case-control studies, but with greater statistical power to detect effects of rare risk factors (Begg and Berwick, 1997). We assessed *MITF* E318K in 1,194 cases and 2,430 controls. It was genotyped by mass spectrometry with the MassArray iPLEX genotyping platform (Sequenom Inc, San Diego, CA). Quality control included use of internal negative controls, sequencing of selected samples to confirm specificity, and agreement between blinded duplicates (Orlow et al., 2012). All statistical tests were two-sided with $P < 0.05$ considered statistically significant. All data were analyzed using SAS 9.3 (Cary, NC). There were 97 carriers of the E138K variant, 44 (3.7%) among the 1,194 cases and 53 (2.2%) among the 2,430 controls. The minor allele frequency was 0.014 among all melanoma patients, similar to other studies noted above.

Presence of *MITF* E318K was significantly associated with risk for melanoma (OR 1.7, 95 % CI 1.1, 2.6, p = 0.02) in a logistic regression model adjusted for the design variables: age at diagnosis, sex, age*sex interaction, and recruitment center. This association is similar in strength to that observed in the Australian and UK studies described above. After further

adjustment for pigmentation characteristics, family history of melanoma, and nevus density, the OR remained as 1.7 (95% CI = 1.1, 2.7, p = 0.03).

Odds ratios for the association of *MITF* E318K with melanoma in categories of other melanoma risk factors (which have been shown to be associated with melanoma in this study (Begg et al., 2006) are shown in Table 1. Increased risk of melanoma with carriage of *MITF* E318K appeared to be strongest among those with traditionally low risk phenotypes: "nonblue" eyes, black/dark brown hair, absence of moles, absence of freckling in youth and absence of a family history of melanoma (Table 1) This effect modification, though, was significant only for mole count and hair color. However, although the interaction is significant, red hair color had an odds ratio of 3.1 (95% CI 0.9, 10.8), so this association needs further study. When evaluating the mole count in relationship to the MITF variant, it can be seen that our results are similar to those reported by Yokoyama et al. (2011), where there is a significant trend among single primary melanomas, our controls, for the association between mole count and the presence of the variant E318K in *MITF* ($p = 0.04$)

The well-established effects of *MC1R* variants on risk of melanoma are similar to those we have found for *MITF* E318K, in being independent of the classical melanoma risk factors. Sturm et al. (2013) suggest the potential for an interaction between *MC1R* "R" variants and the *MITF* variant. However, we have found no evidence of such (either as the traditional "R" variants – D84E, R151C, R160W and D294H – or as these four, including R142H plus stop variants and indels that result in frameshifts and premature stop codons—or as "any" *MC1R* variant) with the presence of the *MITF* variant E318K. We also evaluated the role of such an interaction on pigmentation of the melanoma (amelanotic or pigmented) and found no association. It is likely that both have extra-pigmentary effects that are independent, and stronger in people with dark hair than those with light hair (Demenais et al., 2010; Kanetsky et al., 2011; Egan et al., 2003; Ozolo et al., 2013; Cust et al., 2012). These similarities are consistent with the fact that both genes act in the pathway from UV exposure to eumelanin production. The complexities of this pathway's role in melanomagenesis merit further investigation.

The modification of effects of *MCIR* "R" variants and *MITF* E318K within strata of classical melanoma risk factors suggests that these genetic variants may also be valuable in predicting risk of melanoma in people without classical risk factors and that this information could help in public health and clinical prevention strategies.

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## Significance

These relationships with melanoma risk factors suggest that *MITF* E318K is a genetic risk factor that may be valuable in predicting risk of melanoma in people without classic risk factors for melanoma such as fair hair and multiple nevi and that this information may help with public health and clinical prevention strategies.

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**Table 1**

Odds ratios for the association of the *MITF* E318K variant with melanoma risk in strata of other melanoma risk factors.

| Variables | Strata | Variant Status | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cases | | Controls | | | |
| | | Variant− N (%) | Variant+ N (%) | Variant− N (%) | Variant+ N (%) | Odds Ratio (95% CI)[1] for melanoma associated with *MITF* variant | *P-value for interacti on* |
| **MITF** | All subjects | 1150 (96.3) | 44 (3.7) | 2377 (97.8) | 53 (2.2) | **1.7 (1.1, 2.6)** | NA |
| **Eye color** | Blue | 532 (97.8) | 12 (2.2) | 987 (98.3) | 17 (1.7) | 1.2 (0.5, 2.6) | |
| | Other | 618 (95.1) | 32 (4.9) | 1390 (97.5) | 36 (2.5) | **2.1 (1.2, 3.5)** | 0.25 |
| **Hair Color** | Black/dark brown | 291 (95.7) | 13 (4.3) | 754 (98.6) | 11 (1.4) | **3.8 (1.5, 9.6)** | |
| | light brown/blond | 727 (97.1) | 22 (2.9) | 1393 (97.6) | 35 (2.4) | 1.0 (0.6, 1.8) | |
| | red | 127 (93.4) | 9 (6.6) | 207 (97.6) | 5 (2.4) | 3.1 (0.9, 10.8) | **0.03** |
| **Skin color** | Olive | 92 (97.9) | 2 (2.1) | 320 (98.8) | 4 (1.2) | 1.9 (0.2, 14.2) | |
| | Fair | 793 (96.4) | 30 (3.6) | 1597 (98.2) | 30 (1.8) | **2.2 (1.2, 3.8)** | |
| | Very fair | 263 (95.6) | 12 (4.4) | 450 (95.9) | 19 (4.1) | 0.9 (0.4, 2.0) | 0.23 |
| **Moles** | None | 254 (94.4) | 15 (5.6) | 564 (99.1) | 5 (0.9) | **5.9 (1.9, 18.0)** | |
| | Few | 551 (97.0) | 17 (3.0) | 1207 (97.4) | 32 (2.6) | 1.1 (0.6, 2.1) | |
| | Moderate | 233 (96.7) | 8 (3.3) | 359 (97.5) | 9 (2.5) | 1.5 (0.5, 4.5) | |
| | Many | 75 (96.2) | 3 (3.8) | 114 (96.6) | 4 (3.4) | 1.2 (0.2, 7.0) | <0.01 |
| **Propensity to Burn** | Low | 575 (97.0) | 18 (3.0) | 1319 (98.2) | 24 (2.8) | 1.7 (0.9, 3.4) | |
| | High | 548 (95.8) | 24 (4.2) | 1003 (97.3) | 28 (2.7) | 1.7 (0.9, 3.0) | 0.97 |
| **Inability to Tan** | No | 602 (97.4) | 16 (2.6) | 1377 (98.3) | 24 (1.7) | 1.5 (0.8, 3.1) | |
| | Yes | 521 (95.1) | 27 (4.9) | 945 (97.3) | 26 (2.7) | **1.9 (1.0 3.5)** | 0.62 |
| **Freckling in youth** | None | 451 (95.4) | 22 (4.6) | 1018 (97.8) | 23 (2.2) | **2.3 (1.1, 4.5)** | |
| | Some | 673 (97.1) | 20 (2.9) | 1244 (97.9) | 27 (2.1) | 1.4 (0.7, 2.5) | 0.22 |
| **Family History of Melanoma** | None | 875 (96.2) | 35 (3.8) | 2051 (98.0) | 42 (2.0) | **1.9 (1.2, 3.2)** | |
| | Present | 251 (96.9) | 8 (3.1) | 288 (96.3) | 11 (3.7) | 0.8 (0.3, 2.1) | 0.11 |

| Variables | Strata | Variant Status | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cases | | Controls | | | |
| | | Variant− N (%) | Variant+ N (%) | Variant− N (%) | Variant+ N (%) | Odds Ratio (95% CI)[1] for melanoma associated with *MITF* variant | *P-value for interacti on* |
| **Personal History of NMSC** | None | 588 (96.5) | 21 (3.5) | 1814 (97.9) | 38 (2.1) | 1.5 (0.7, 3.1) | |
| | Present | 541 (96.1) | 22 (3.9) | 536 (97.3) | 15 (2.7) | 1.7 (0.9, 3.0) | 0.96 |
| **MC1R** | No MC1R variant | 146 (98.0) | 3 (2.0) | 363 (98.4) | 6 (1.6) | 1.09 (0.24, 4.99) | |
| | Any MCIR variant | 903 (96.1) | 37 (3.9) | 1770 (97.6) | 44 (2.4) | 1.65 (1.01, 2.69) | 0.55 |

[1] Odds ratio and 95% CI adjusted for age, sex, center and age*sex interaction.