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Exploring the Germline Genetics of In Situ and Invasive Cutaneous Melanoma

A Genome-Wide Association Study Meta-Analysis

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IMPORTANCE It is unknown whether germline genetic factors influence in situ melanoma risk differently than invasive melanoma risk.

OBJECTIVE To determine whether differences in risk of in situ melanoma and invasive melanoma are heritable.

DESIGN, SETTING, AND PARTICIPANTS Three genome-wide association study meta-analyses were conducted of in situ melanoma vs controls, invasive melanoma vs controls, and in situ vs invasive melanoma (case-case) using 4 population-based genetic cohorts: the UK Biobank, the FinnGen cohort, the QSkin Sun and Health Study, and the Queensland Study of Melanoma: Environmental and Genetic Associations (Q-MEGA). Melanoma status was determined using *International Statistical Classification of Diseases and Related Health Problems* codes from cancer registry data. Data were collected from 1987 to 2022, and data were analyzed from September 2022 to June 2023.

EXPOSURE In situ and invasive cutaneous melanoma.

MAIN OUTCOMES AND MEASURES To test whether in situ and invasive melanoma have independent heritable components, genetic effect estimates were calculated for single-nucleotide variants (SNV; formerly single-nucleotide polymorphisms) throughout the genome for each melanoma. Then, SNV-based heritability was estimated, the genetic correlation between melanoma subtypes was assessed, and polygenic risk scores (PRS) were generated for in situ vs invasive status in Q-MEGA participants.

RESULTS A total of 6 genome-wide significant loci associated with in situ melanoma and 18 loci with invasive melanoma were identified. A strong genetic correlation (genetic r = 0.96; 95% CI, 0.76-1.15) was observed between the 2 classifications. Notably, loci near *IRF4*, *KLF4*, and *HULC* had significantly larger effects for in situ melanoma compared with invasive melanoma, while *MC1R* had a significantly larger effect on invasive melanoma compared with in situ melanoma. Heritability estimates were consistent for both, with in situ melanoma heritability of 6.7% (95% CI, 4.1-9.3) and invasive melanoma heritability of 4.9% (95% CI, 2.8-7.2). Finally, a PRS, derived from comparing invasive melanoma with in situ melanoma genetic risk, was on average significantly higher in participants with invasive melanoma (odds ratio per 1-SD increase in PRS, 1.43; 95% CI, 1.16-1.77).

CONCLUSIONS AND RELEVANCE There is much shared genetic architecture between in situ melanoma and invasive melanoma. Despite indistinguishable heritability estimates between the melanoma classifications, PRS suggest germline genetics may influence whether a person gets in situ melanoma or invasive melanoma. PRS could potentially help stratify populations based on invasive melanoma risk, informing future screening programs without exacerbating the current burden of melanoma overdiagnosis.

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Supplemental content

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In situ melanoma is a cancer of the melanocytes that has grown superficially along the avascular epidermal layer of the skin but has not invaded deeper into the dermis. An invasive melanoma is a more aggressive cancer that has broken through to the dermal layer of the skin, where it has the chance to metastasize.[1] In situ melanoma can be a precursor to invasive melanoma.[2] However, a 2022 study suggests that not all in situ lesions are destined to become invasive, and many will remain indolent.[3]

Five-year survival following diagnosis of invasive melanoma ranges from 93% for those diagnosed at stage 1 to 53% for those diagnosed at stage 4,[4] highlighting the importance of early detection for prognosis. Trends over the past 50 years show that in situ diagnoses are rising faster than invasive lesions—a sign of melanoma overdiagnosis.[3,5-7] Furthermore, screening for melanoma has been shown to primarily lead to the detection of in situ rather than invasive lesions,[8] which calls into question the feasibility of melanoma screening as a method of early detection of invasive melanoma. Distinguishing between a person's risk of benign in situ melanoma and their risk of invasive melanoma could have clinical benefits in the future by helping prioritize screening for people with a high risk of faster-growing invasive melanoma.

The role of germline genetic variation on melanoma risk is well studied, with a total of 55 replicable genetic loci associated with melanoma risk.[9,10] However, it remains unclear whether germline genetics influence the risk of melanoma stage 0 (in situ) vs the risk of melanoma stages 1-4 (invasive). Polygenic risk scores (PRS) could potentially stratify people by their risk of developing invasive melanoma as opposed to the more broad melanoma definition including both in situ and invasive disease. These PRS could inform future melanoma screening practices but not exacerbate the burden of overdiagnosis.

Using data from the UK Biobank (UKBB),[11] the FinnGen cohort,[12] the QSkin Sun and Health Study (QSkin),[13] and the Queensland Study of Melanoma: Environmental and Genetic Associations (Q-MEGA),[14] we explored if (1) there are differences between the genetic loci associated with diagnosis of an in situ or invasive cutaneous melanoma; (2) propensity to develop in situ vs invasive cutaneous melanoma is heritable; and (3) we can construct a PRS that is associated with being diagnosed with an in situ vs an invasive cutaneous melanoma.

Key Points

Question Does germline genetic variation influence the risk of a person developing an in situ or an invasive melanoma?

Findings In this genome-wide association study meta-analysis of in situ and invasive melanoma risk, there was much shared genetic architecture influencing both melanoma classifications. However, polygenic risk scores (PRS) reflecting the genetic difference between the 2 classifications were significantly higher in invasive melanoma groups than in situ melanoma groups, suggesting germline genetics plays a role in the differential melanoma diagnosis.

Meaning PRS may be used to stratify people based on their risk of invasive melanoma, which could inform future melanoma screening practices without exacerbating melanoma overdiagnosis.

Methods

Cohort Description

Case status for in situ and invasive melanoma across cohorts was determined through cancer registry records. The **Table** shows sample sizes and diagnosis codes defining each melanoma status within each cohort. In UKBB, QSkin, and Q-MEGA, individuals with both in situ and invasive melanoma were classified as invasive cases only, not in situ cases. The FinnGen cohort had a 5.2% overlap of in situ melanoma with invasive melanoma. Although unavoidable, the small overlap is expected to have minimal impact on the meta-analysis results. The Q-MEGA cohort includes samples from the Digestive Health Study[15] and singletons from the Brisbane Adolescent Twin study.[16] These samples were collected at a similar time and analyzed under the same conditions as the Q-MEGA samples

To avoid bias through population stratification, all cohorts were restricted to participants of European ancestry using the first 2 genetic principal components compared with a European reference population. For UKBB, the reference population was a self-reported White British population; for FinnGen, a homogenous Finnish population of European descent was used; and for QSkin and Q-MEGA, a European HapMap reference population was used. A thorough cohort description can be found in the eMethods in Supplement 1.

Genome-Wide Association Study

Genome-wide association studies (GWAS) perform logistic regression to assess associations between common single-nucleotide variant (SNV; formerly single-nucleotide polymorphism) genotypes and melanoma status. For the UKBB, QSkin, and Q-MEGA cohorts, 3 GWAS were performed: 2 case-control analyses (in situ or invasive melanoma vs melanoma-free controls) and 1 case-case analysis (in situ vs invasive melanoma risk). The FinnGen cohort supplied summary GWAS data for in situ melanoma and invasive melanoma vs controls, GWAS results for in situ melanoma vs invasive melanoma were unavailable. Details of GWAS software, covariates, and how sample relatedness was controlled for can be found in eTable 1 in Supplement 1. Details of sample and genotype quality control procedures can be found in eTable 2 in Supplement 1. A 2-tailed P value cutoff for genome-wide significance was set at $P < 5 \times 10^{-8}$.

Meta-Analysis

METAL version 1.0 (University of Michigan) was used to meta-analyze the results for each cohort, using effect estimates (log odds ratio) and standard errors from the Q-MEGA, QSkin, and UKBB cohorts for the case-case GWAS, and the addition of the FinnGen cohort for case-control analysis. SNVs were only included in the meta-analysis results if they were present in at least 2 of the input GWAS.

Table. Invasive, In Situ, and Control Phenotype Definition for Each Cohort

Cohort	In situ melanoma			Invasive melanoma			Controls	
	Data source	Code	Patients, No.	Data source	Code	Patients, No.	Definition	Controls, No.
UK Biobank	Cancer registry; Summary diagnoses	*ICD-10* code D03	1818	Cancer registry; Summary diagnoses	*ICD-9* codes 1720-1728; *ICD-10* code C43	5754	Remaining participants excluding an in situ or invasive melanoma diagnosis	430 968
FinnGen	Hospital discharge; Cause of death	*ICD-10* code D03	887	Cause of death; Cancer registry	*ICD-8* and *ICD-9* code 172; *ICD-10* code C43; *ICD-O* codes C44.0-C44.9; Morphology codes 8720, 8721, 8726, 8730, 8742, 8743, 8744, and 8745; Behavior code 3	2993	Remaining participants excluding an in situ or invasive melanoma diagnosis or a diagnosis of any other cancers	286 876 and 287 137
QSkin	Queensland cancer registry	*ICD-O* codes C44.0-C44.9; Morphology codes 87200-87900; Behavior code 2	586	Queensland cancer registry	*ICD-O* codes C44.0-C44.9; Morphology codes 87200-87900; Behavior code 3	668	Remaining participants excluding an in situ or invasive melanoma diagnosis	15 309
Q-MEGA (OMNI array)	Queensland cancer registry	*ICD-O* codes C44.0-C44.9; Morphology codes 87200-87900; Behavior code 2	125	Queensland cancer registry	*ICD-O* codes C44.0-C44.9; Morphology codes 87200-87900; Behavior code 3	530	Digestive Health Study participants excluding an in situ or invasive melanoma diagnosis	541
Q-MEGA (610K array)	Queensland cancer registry	*ICD-O* codes C44.0-C44.9; Morphology codes 87200-87900; Behavior code 2	148	Queensland cancer registry	*ICD-O* codes C44.0-C44.9; Morphology codes 87200-87900; Behavior code 3	607	Singletons from the Brisbane Adolescent Twin Study excluding an in situ or invasive melanoma diagnosis	1239

Abbreviations: *ICD-8*, *International Classification of Diseases, Eighth Revision*; *ICD-9*, *International Classification of Diseases, Ninth Revision*; *ICD-10*, *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*; *ICD-O*, *International Classification of Disease for Oncology*; Q-MEGA, Queensland Study of Melanoma: Environmental and Genetic Associations; QSkin, QSkin Sun and Health Study.

Heritability and Genetic Correlation

Linkage disequilibrium score regression (LDSC) was used to estimate the SNV-based heritability of each GWAS meta-analysis performed.[17] An additional function of LDSC was used to leverage linkage disequilibrium patterns across the genome to calculate the genetic correlation between the in situ and invasive GWAS meta-analysis.[18] A 2-tailed *P* value was considered significant if it was less than .05. More details of LDSC can be found in the eMethods in Supplement 1.

Statistical Analysis

Effect Size Comparisons

The effect estimates of 90 independent melanoma-associated SNVs (previously reported[9]) were extracted from the results of the GWAS meta-analyses of in situ and invasive melanoma and compared using a Pearson correlation test in R version 4.0.2 (The R Foundation). Additionally, the effect estimates of the lead SNVs of the in situ and invasive melanoma meta-analyses were compared using a Pearson correlation test. To determine if individual SNV effect estimates significantly differed between in situ and invasive melanoma, a χ^2 test was performed using the equation found in the eMethods in Supplement 1. A Bonferroni-corrected 2-tailed *P* value significance cutoff adjusting for the number of SNVs was set at $P < .003$.

PRS

A leave-one-cohort-out approach applied to the in situ vs invasive melanoma meta-analysis excluded part of the Q-MEGA participants who were genotyped on the OMNI-Quad array (125 with in situ melanoma and 530 with invasive melanoma). We used clumping and thresholding in PLINK version 1.9 to generate PRS in the excluded Q-MEGA sample. The clumping threshold for inclusion of SNVs into the model was set at $r^2 < 0.01$, 1000 kilobases, and the following *P* value thresholds: 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4}, 1×10^{-3}, .01, .10, .50, and <.99. A 2-tailed *P* value was considered significant at $P < .005$, accounting for the 10 PRS thresholds tested.

PRS were transformed to assume a normal distribution, the mean of which was set to zero. A logistic regression of melanoma status on melanoma PRS with age, sex, and ancestral principal components 1 to 10 as covariates was conducted in R version 4.0.2 to assess the PRS ability to distinguish Q-MEGA participants' risk of invasive melanoma.

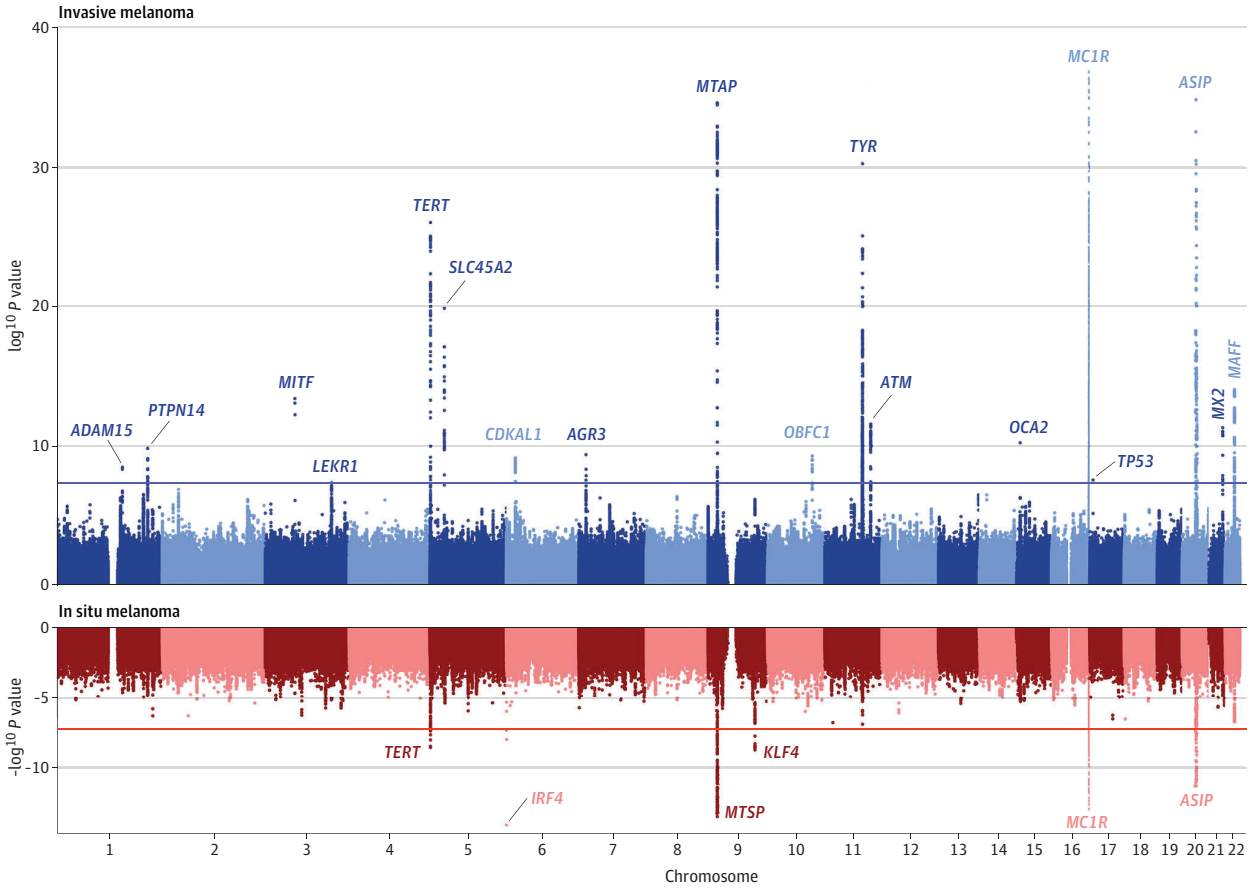
Moles

Moles data were derived from the QSkin cohort to determine whether those with in situ melanoma or invasive melanoma had significantly more moles, which was assessed with a Mann-Whitney-Wilcoxon test. A 2-tailed *P* value was significant at $P < .05$. See the eMethods in Supplement 1 for more details.

Results

The GWAS meta-analysis of invasive melanoma vs controls identified 18 independent genome-wide significant loci (linkage disequilibrium $r^2 < 0.01$; distance >1000 kilobase pairs [kb] apart; $P < 5 \times 10^{-8}$) (**Figure 1**; eTable 3 in Supplement 1). All 18

Figure 1. Miami Plot of Invasive Melanoma and In Situ Melanoma



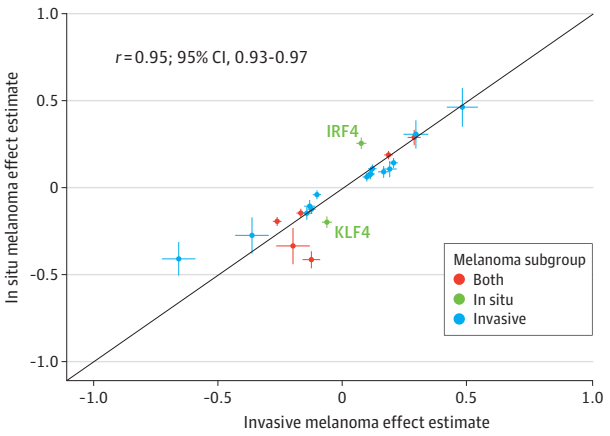
A Miami plot showing *P* values from the genome-wide association study meta-analysis of invasive melanoma vs controls on a $\log_{10}$ scale, and in situ melanoma vs controls on a $-\log_{10}$ scale. The plot has been capped at a $-\log_{10}$ *P* value less than 30. The samples used in the genome-wide association study meta-analysis were the UK Biobank, FinnGen, QSkin Sun and Health Study, and Queensland Study of Melanoma: Environmental and Genetic Associations. To be present in the plot, a given single-nucleotide variant had to be present in at least 2 of the meta-analyzed cohorts. For a Miami plot of the meta-analysis results with all analyzed single-nucleotide variant plotted, regardless of how many cohorts they were present in, please see eFigure 1 in Supplement 1. The main difference between these figures is that eFigure 1 in Supplement 1 shows an additional locus on chromosome 1 (*KISS1*) for invasive melanoma, which was only present in the FinnGen population.

loci have been previously shown to be associated with cutaneous melanoma.[9] The GWAS meta-analysis of in situ melanoma vs controls revealed 6 independent genome-wide significant loci (Figure 1; eTable 4 in Supplement 1). Of these, 4 (*TERT*, *MTAP*, *MC1R*, and *ASIP*) overlapped with invasive melanoma. Two loci, *IRF4* (rs12203592; chromosome 6) and *KLF4* (rs10979147; chromosome 9) reached genome-wide significance for in situ melanoma but not for invasive melanoma (eFigures 2 and 3 in Supplement 1). These 2 SNVs had significantly stronger effects on in situ melanoma than invasive melanoma (eTable 4 in Supplement 1). In addition to melanoma risk, the *IRF4* and *KLF4* loci have also both been shown to be associated with nevus count—a strong risk factor for melanoma.[9,19] A Mann-Whitney-Wilcoxon test revealed marginally higher mole counts in people with invasive melanoma compared with those with in situ melanoma within the QSkin cohort.

A comparison of in situ and invasive melanoma effect estimates using 90 lead SNVs associated with melanoma risk from Landi et al[9] showed a strong correlation between in situ and invasive melanoma effect estimates (r = 0.91; 95% CI, 0.89-0.93) (eFigure 5 in Supplement 1). Similarly, comparing the effect estimates of the lead SNVs from the in situ and invasive melanoma meta-analyses revealed a correlation of 0.95 (95% CI, 0.93-0.97) (**Figure 2**). The genetic correlation between the in situ and invasive melanoma GWAS meta-analysis was 0.96 (95% CI, 0.76-1.15). Similarly, SNV heritability estimates for in situ melanoma were not distinguishable from that of invasive melanoma; in situ melanoma heritability was 6.7% (95% CI, 4.1-9.3) and invasive melanoma heritability was 4.9% (95% CI, 2.8-7.2).

The case-case GWAS meta-analysis of in situ vs invasive melanoma (**Figure 3**) revealed 1 genome-wide significant SNV on chromosome 6 (rs4566922). Investigating the rs4566922 SNV on the University of California, Santa Cruz genome browser[20] reveals it is located in a noncoding area of DNA that has been conserved throughout evolution, suggesting it may be functionally relevant. However, a functional role for this SNV

Figure 2. Scatterplot of Effect Estimates of Lead Loci of In Situ and Invasive Melanoma



Effect size plot of the lead single-nucleotide variants from the in situ melanoma and the invasive melanoma genome-wide association study meta-analysis. Point colors represent the genome-wide significance of the point (ie, whether the single-nucleotide variant was significant in the in situ melanoma analysis, invasive melanoma analysis, or both analyses). Effect estimates for the *IRF4* and *KLF4* were significantly different from one another at $P < .003$. The solid black line represents a correlation of 1, and the crosses represent standard errors of the genome-wide association study effect estimates.

has not yet been assigned. There are 3 genes identified within a 500-kb window on either side of the SNV; these are *HULC* (217.6 kb), *LOC100506207* (86.04 kb), and *SLC35B3* (435.9 kb). This SNV is also not in high linkage disequilibrium with other SNVs in the region, due to a high level of recombination in the surrounding area (eFigure 4 in Supplement 1). LDSC of the case-case GWAS produced no meaningful heritability estimate, despite a mean χ^2 SNVs of 1.021.

A PRS was generated in 655 Q-MEGA participants who were excluded from the main case-case GWAS meta-analysis. The PRS was constructed using 64 497 independent SNV effect estimates that had a *P* value threshold less than .10 in the case-case GWAS meta-analysis. The mean PRS for people with invasive melanoma in the Q-MEGA subset was significantly higher than that of participants with in situ melanoma, with increased odds for invasive melanoma (odds ratio per 1-SD increase in PRS, 1.43; 95% CI, 1.16-1.77) (**Figure 4**). The robustness of this PRS was further tested by removing the 3 SNVs that showed a different effect estimate for in situ and invasive melanoma—rs4566922 (*HULC*), rs12203592 (*IRF4*), and rs10979147 (*KLF4*)—which revealed little change to the results (odds ratio, 1.42; 95% CI, 1.15-1.75). All PRS *P* value thresholds showed a similar correlation (eTable 5 in Supplement 1).

Discussion

The SNV heritability estimates for in situ and invasive melanoma suggest the amount that variation in SNV effects influences variation in melanoma risk is comparable between the 2 melanoma subtypes. Many of the genome-wide significant loci of the GWAS meta-analyses of in situ and invasive melanoma overlap, with consistent SNV effect estimates between the 2 melanoma diagnoses. Additionally, there is a strong genetic correlation between the 2 types of melanoma. Overall, this suggests a large amount of the common genetic variation influencing the 2 melanoma classifications is shared.

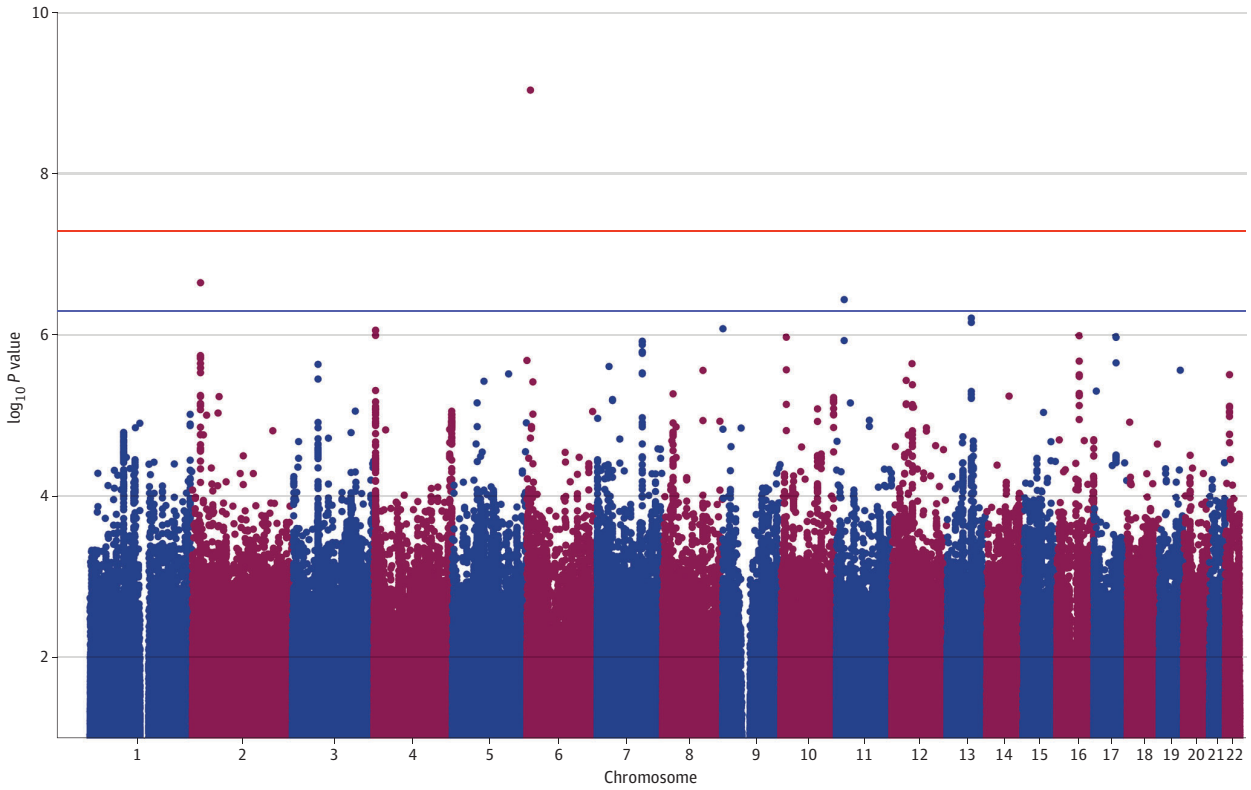
Although the genetic architecture of in situ and invasive melanoma risk appears largely shared, 2 loci, rs10979147 (near *KLF4*) and rs12203592 (near *IRF4*), show significantly larger effects on in situ melanoma risk compared with invasive melanoma risk. These loci were genome-wide significant only for in situ melanoma, despite the GWAS meta-analysis of invasive melanoma having higher statistical power to detect loci. Both *IRF4* and *KLF4* have been previously associated with melanoma risk, although the individual SNVs show contradictory evidence for an association with melanoma in previous analyses.[9] In particular, rs12203592 exhibited a high heterogeneity between meta-analyzed cohorts in the study by Landi et al[9] (I^2 = 83%; fixed-effect meta-analysis *P* value = 7×10^{-13}; random effects = 0.11).

IRF4 and *KLF4* have both previously been associated with mole count,[19,21] which may explain the differential effects of these loci on the 2 melanoma subtypes observed here. Misdiagnosis of dysplastic moles as in situ melanoma could introduce bias through inaccurate phenotyping. Alternatively, people with higher average mole count may undergo more frequent melanoma screenings, increasing the likelihood of detecting an in situ melanoma as opposed to an invasive melanoma,[3,8] though this is unlikely to be caused by the small effect of *IRF4* and *KLF4* alone. For in situ melanoma, the direction of effect for these 2 loci is consistent with their effect on nevus count,[19] indicating that the same alleles increase both mole counts and in situ melanoma risk. However, a Mann-Whitney-Wilcoxon test within the QSkin cohort revealed slightly (though significantly) higher mole counts in the invasive melanoma group than the in situ melanoma group, contradicting the hypothesis that in situ melanoma risk is influenced by mole count.

The rs12203592*C/T SNV is located in an enhancer of *IRF4,* which encodes interferon regulatory factor 4, a transcription factor expressed in immune cells and melanocytes.[22-24] Both alleles of rs12203592 have been associated with increased melanoma risk due to diverging effects on solar-associated melanoma vs nevus-associated melanoma.[21,25-27] rs12203592*T reduced IRF4 levels and is associated with reduced expression of tyrosinase, causing lighter eye and skin color and increased mole and freckling density.[23,28] Despite this association, the action of *IRF4* on mole and melanoma development remains unclear. However, it has been suggested that differential IRF4 levels help melanocytes evade immune detection after UV damage, increasing the mutational burden of the melanocytes.[29]

KLF4 encodes Krüppel-like factor 4, which plays a crucial role in many cancers, due to its ability to induce cell cycle arrest and also block apoptosis.[30] Augmented *KLF4* expression has been observed in many melanoma cell lines. Increased KLF4 downregulates p53, reducing *CDKN1A*-mediated cell-cycle arrest. It also upregulates antiapoptotic factors BCL-2 and BCL-XL.[31,32] The reduced apoptotic capacity of melanocytes with increased *KLF4* expression has been

Figure 3. Manhattan Plot Case-Case Genome-Wide Association Study Meta-Analysis of In Situ Melanoma vs Invasive Melanoma



This Manhattan plot from the genome-wide association study meta-analysis of in situ vs invasive melanoma used data from the UK Biobank, QSkin Sun and Health Study, and Queensland Study of Melanoma: Environmental and Genetic Associations. The red line indicates genome-wide significance ($P < 5 \times 10^{-8}$) on the $\log_{10}$ scale, and the blue line indicates suggestive genome-wide significance ($P < 5 \times 10^{-7}$). The single genome-wide significant single-nucleotide variant is rs4566922. This single-nucleotide variant is near to the gene *HULC*.

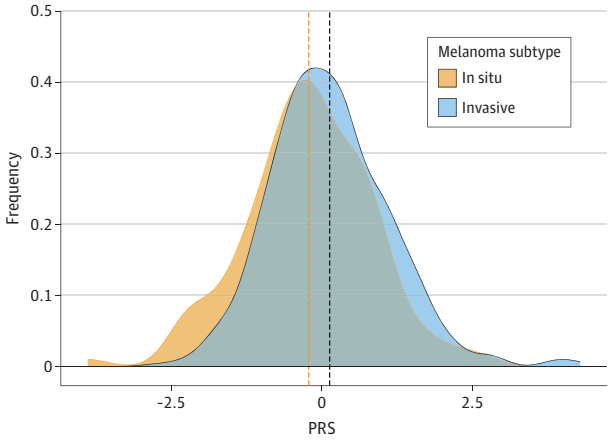
linked with sustained oncogenic BRAF effects in melanoma cell lines in vitro.[32] In moles, augmented BRAF levels are believed to trigger the proliferation of melanocytes until the other cell-cycle checkpoints prevent further proliferation.[33] Potentially, this KLF4-mediated elongation of the oncogenic effect of BRAF within melanocytes allows continued cell proliferation beyond mole formation to melanoma in situ, which may explain why we observe fewer moles in the in situ melanoma group compared with the invasive melanoma group.

The invasive melanoma vs control analysis identified 18 loci, 3 of which (*SLC45A2*, *AGR3*, and *MC1R*) had a significantly larger effect on invasive melanoma risk than in situ melanoma risk; *MC1R* was the only locus to remain with a significantly different effect after correcting for multiple testing (eTable 3 in Supplement 1). It is conceivable that these 3 genes pose a greater risk for invasive melanoma than melanoma in situ, and therefore carriers of 1 or more alleles of these genes might have an increased risk of invasive melanoma, despite *MC1R* having a genome-wide significant effect on both in situ and invasive melanoma. Further analysis using a larger sample size is required to confirm these findings.

The case-case analysis (in situ vs invasive melanoma) had substantially lower power to detect genome-wide significant effects than the 2 case-control analyses; this is partly due to the omission of the FinnGen cohort from the case-case meta-analysis but also by having a smaller control set (Table). The GWAS revealed 1 significant SNV (rs4566922); however, it is difficult to identify a potential causal gene linked to this locus. To our knowledge, there is no in-silico evidence linking this variant to a gene function. The nearest gene to this SNV (*LOC100506207*) encodes a poorly characterized long noncoding RNA. The second nearest gene (*HULC*) encodes a long noncoding RNA with an oncogenic effect in hepatocarcinoma[34]; however, this gene has limited expression in the skin, making it a less plausible functional target. The third closest gene (almost 500 kb away) is *SLC35B3*, which encodes a solute carrier and has no previous association with melanoma or its risk factors. Two other nominally significant ($P < 5 \times 10^{-6}$) loci were found in the case-case GWAS meta-analysis: rs12710704 (near gene *OSR1*) and rs139602343 (near gene *MRGPRX1*). However, neither of these loci have previously been associated with traits related to the skin.

The SNV heritability estimate for the case-case GWAS meta-analysis was inconclusive, with an estimate greater than 100% and 95% CIs that cross zero. This may mean that the difference between in situ and invasive melanoma risk is not heritable. However, the low power of the case-case analysis is a more plausible explanation, as LDSC requires a minimum mean

Figure 4. Comparison of the In Situ Melanoma vs Invasive Melanoma Polygenic Risk Score (PRS) Distribution Between Individuals With In Situ and Invasive Melanoma in the Q-MEGA Sample



Comparison of the distribution of the PRS of the Queensland Study of Melanoma: Environmental and Genetic Associations (Q-MEGA) sample. The vertical dotted lines represents the mean PRS for each group. The PRS is weighted using the effect estimates derived from the case-case genome-wide association study meta-analysis of in situ vs invasive melanoma in the UK Biobank, QSkin Sun and Health Study, and part of Q-MEGA.

χ^2 across all SNVs greater than 1.02; our case-case GWAS only just passed this threshold at 1.021.[17] As the case-case GWAS meta-analysis assesses the genetic difference between in situ and invasive melanoma risk, a PRS generated from the effect estimates is conceivably more sensitive to reflect genetic differences between the 2 melanoma subtypes. The mean PRS of people with invasive melanoma was significantly higher than those with in situ melanoma and people without melanoma (eFigure 6 in Supplement 1). This was seen with and without the inclusion of *KLF4, IRF4*, and *HULC* effect estimates in the model. This suggests that the risk of invasive compared with in situ melanoma may be heritable and recognizes that the genes may be acting in synergy to overall increase a person's risk of one type of melanoma over the other. This suggests that PRS has the potential to stratify a population by invasive melanoma risk, which could benefit future melanoma screening programs by improving melanoma risk prediction models' specificity to invasive melanoma risk. Nevertheless, additional research is needed to assess (1) the feasibility of melanoma screening programs and (2) the practicality of integrating invasive melanoma–specific PRS to enhance predictive accuracy.

Limitations

This study has limitations. First, 5.22% of in situ melanoma cases in the FinnGen cohort also have an invasive melanoma diagnosis due to the cohort's phenotype definition. Second, we cannot determine the proportion of fast-growing in situ melanoma cases. Lack of natural history of melanoma data reduces power to detect genetic differences. Additionally, pathology-confirmed histology is unavailable, potentially leading to misclassifications of the moles and melanoma. For example, approximately 30% of patients diagnosed with melanoma in situ have invasive melanoma if the pathology is reassessed.[35] Moreover, a larger sample size is needed for meaningful heritability estimates and genetic locus detection. We advise caution when interpreting the results as independent cohort replication, and future biological validation, such as protein staining of IRF4 and KLF4, is required for GWAS findings.

Conclusions

In summary, there is a substantial shared genetic component between in situ melanoma and invasive melanoma risk, with many overlapping genome-wide significant loci. However, loci near *IRF4* and *KLF4* may have a stronger influence on in situ melanoma risk, while *MC1R*, *SLC45A2*, and *AGR3* may have a greater impact on invasive melanoma. The SNV rs4566922 may also highlight an area of the genome that influences differential melanoma diagnosis, yet a functional role remains undefined. A significantly higher mean PRS in people with invasive compared with in situ melanoma suggests that germline genetic variation plays a role in differential melanoma diagnosis. If PRS are to be used to inform future targeted melanoma screening programs, this will allow increased prediction specificity toward invasive melanoma, which will not exacerbate the current burden of melanoma overdiagnosis. However, future work to replicate these findings and assess the feasibility of a targeted melanoma screening program should be conducted.

ARTICLE INFORMATION

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Author Contributions: Mr Ingold had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Ingold, Seviiri, Neale, Whiteman, Hayward, Montgomery, MacGregor, Law.
Acquisition, analysis, or interpretation of data: Ingold, Seviiri, Ong, Pandeya, Whiteman, Olsen, Martin, Duffy, Khosrotehrani, Montgomery, MacGregor, Law.
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