Multi-Trait Genetic Analysis Identifies Autoimmune Loci Associated with Cutaneous Melanoma

JID Open

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Genome-wide association studies (GWAS) have identified a number of risk loci for cutaneous melanoma. Cutaneous melanoma shares overlapping genetic risk (genetic correlation) with a number of other traits, including its risk factors such as sunburn propensity. This genetic correlation can be exploited to identify additional cutaneous melanoma risk loci by multitrait analysis of GWAS (MTAG). We used bivariate linkage disequilibrium−score regression score regression to identify traits that are genetically correlated with clinically confirmed cutaneous melanoma and then used publicly available GWAS for these traits in a multitrait analysis of GWAS. Multitrait analysis of GWAS allows GWAS to be combined while accounting for sample overlap and incomplete genetic correlation. We identified a total of 74 genome-wide independent loci, 19 of them were not previously reported in the input cutaneous melanoma GWAS meta-analysis. Of these loci, 55 were replicated ($P < 0.05/74$, Bonferroni-corrected P-value in two independent cutaneous melanoma replication cohorts from Melanoma Institute Australia and 23andMe, Inc. Among the, to our knowledge, previously unreported cutaneous melanoma loci are ones that have also been associated with autoimmune traits including rs715199 near *LPP* and rs10858023 near *AP4B1*. Our analysis indicates genetic correlation between traits can be leveraged to identify new risk genes for cutaneous melanoma.

Journal of Investigative Dermatology (2022) **142,** 1607−1616; doi:10.1016/j.jid.2021.08.449

INTRODUCTION

Cutaneous melanoma (hereafter melanoma) is the most aggressive form of skin cancer; while representing only 2% of skin cancers diagnosed, 80% of deaths due to skin cancers are due to melanoma (Linos et al., 2009; US Preventive Services Task Force et al., 2016). In 2019 in Australia alone, there were over 15,000 cases of invasive melanoma and 1,700 deaths (Australian Institute of Health and Wlefare, 2019), while in the United States, there were over 97,000 cases and 7,000 deaths (American Cancer Society, 2019). Twin studies have estimated the heritability of melanoma to be 58% (95% confidence interval [CI], 43−73%) (Mucci et al., 2016). Depending on the approach and the dataset used, the single nucleotide polymorphism (SNP) heritability

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Abbreviations: HWE, Hardy-Weinberg equilibrium; IBD, identity by descent; KC, keratinocyte cancers; LD, linkage disequilibrium; LDSC, linkage disequilibrium score regression; MAF, minor allele frequency; MIA, Melanoma Institute Australia; MTAG, multi-trait analysis of GWAS; PC, principal components; RA, rheumatoid arthritis; r_g, genetic correlation; UKBB, UK Biobank

Received 3 March 2021; revised 23 August 2021; accepted 25 August 2021; accepted manuscript published online 20 November 2021; corrected proof published online 24 March 2022

Table 1. Sample Sizes, Correlation with Melanoma, and LD Score Analysis of GWAS Datasets

Trait	Cases or *Total N for Linear GWAS	Controls	r_g[1]	SE[2]	*P*[3]
KC (UKBB)[4]	18,538	340,302	0.46	0.088	2.0×10^{-7}
Skin burn type (QSkin)[4]	*16,169	—	0.64	0.123	2.1×10^{-7}
Mole count (Duffy et al., 2018)[4]	*65,777	—	0.57	0.113	3.4×10^{-7}
Potassium in urine (UKBB)[4]	*425,892	—	0.13	0.034	1.0×10^{-4}
Skin color (QSkin)[4]	*16,185	—	−0.56	0.149	2.0×10^{-4}
Rheumatoid arthritis (Okada et al., 2014)[4]	14,361	43,923	−0.21	0.057	2.0×10^{-4}
KC (QSkin)[4]	8,145	4,797	0.48	0.129	2.0×10^{-4}
Skin tan type (QSkin)[4]	*16,141	—	−0.55	0.160	5.0×10^{-4}
Red hair (QSkin)	973	15,202	0.48	0.150	1.5×10^{-3}
Vitamin D (Blood) (UKBB)	*401,529	—	0.10	0.033	1.6×10^{-3}
Hair color exc. Red (UKBB)	*411,967	—	−0.27	0.084	1.6×10^{-3}
Childhood sunburns (UKBB)	*331,020	—	0.37	0.128	3.5×10^{-3}
Skin color (UKBB)	*433,288	—	−0.34	0.123	5.8×10^{-3}
Cr (enzymatic) in urine (UKBB)	*426,794	—	0.08	0.029	8.6×10^{-3}
Leukocyte count (UKBB)	*426,772	—	−0.06	0.025	0.011
Hypothyroidism (Zhou et al., 2018)	14,871	391,429	0.10	0.042	0.012
Skin burn type (UKBB)	*430,447	—	−0.30	0.123	0.014
Total protein (UKBB)	*384,728	—	−0.09	0.038	0.019
Risky behavior (Karlsson Linnér et al., 2019)	*466,571	—	0.09	0.040	0.020
Hair color except for red (QSkin)	*15,202	—	−0.29	0.133	0.033
ALP (blood) (UKBB)	*419,535	—	−0.06	0.028	0.036
KC cancer (eMERGE)	1,565	8,756	0.27	0.139	0.048

Abbreviations: ALP, alkaline phosphatase; Cr, creatinine; KC, keratinocyte; SE, standard error; UKBB, United Kingdom Biobank; WBC count, white blood cell count.
[1]r_g = genetic correlation with melanoma.
[2]SE of the r_g.
[3]*P*-value of r_g estimate.
[4]Traits included in the secondary sensitivity analysis *P*-value < 0.00091 (0.05 corrected for the 55 GWAS).

measured using melanoma genome-wide association study (GWAS) data ranges from 12 to 30% (Landi et al., 2020; Lu et al., 2014; Yang et al., 2011; Zhang et al., 2018), reinforcing that there is still a large proportion of melanoma heritability to be discovered. One approach to identify additional genes associated with melanoma is to increase statistical power by leveraging its (genetic) correlation with other traits. If the same SNPs are associated with both traits, then a combined analysis will have greater statistical power.

A number of traits that are themselves polygenic have been shown to be correlated with risk of melanoma. Having many moles is the strongest single risk factor for melanoma, and combining mole count and melanoma GWAS data has identified additional loci (Duffy et al., 2018; Gandini et al., 2005; Landi et al., 2020). Ultraviolet radiation (UVR) is the main environmental risk factor for all skin cancers. As a result, melanoma and the keratinocyte cancers (KCs), basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are more frequent in people with traits associated with sensitivity to the effects of UVR exposure such as fair skin and propensity to sunburn. These skin pigmentation–related traits are themselves under genetic control (Pavan and Sturm, 2019). Given this, it is unsurprising that several genetic variants and genes common to all skin cancers are involved in regulating human pigmentation, for example, *MC1R*, *IRF4*, and *SLC45A2* (Gerstenblith et al., 2010; Roberts et al., 2019). The importance of UVR exposure suggests that other traits may share genetic risk with melanoma. UVR exposure is also important for vitamin D synthesis, and higher levels of serum vitamin D have been associated with an increased risk of melanoma in observational studies (Mahamat-Saleh et al., 2020). While our recent Mendelian randomization analysis suggests that vitamin D levels themselves may not be causal for melanoma (Liyanage et al., 2020), that does not rule out a genetic overlap with melanoma with respect to pigmentation and sun exposure. The involvement of pleiotropic genes such as *CASP8*, *CYP1B1*, and *TERT* among skin cancers suggests a shared genetic architecture that cannot be wholly explained by a common effect of pigmentation. Finally, although the exact relationship/underlying mechanism between a dysregulated immune system and risk of skin cancer has not yet been explored extensively, autoimmune conditions, including psoriasis are known to be associated with melanoma (Bhattacharya et al., 2016).

We hypothesized that a combined analysis of GWAS of traits genetically correlated with melanoma will identify additional risk loci for melanoma. The utility of a traditional meta-analysis is limited when genetic correlation (r_g) is incomplete (i.e., much less than 1) and also where there is sample overlap in contributing GWAS. To address this, we have employed the multitrait analysis of GWAS (MTAG) method (Turley et al., 2019). MTAG provides accurate SNP effect size estimates even with overlapping samples and incomplete r_g. As the gain in statistical power for melanoma gene discovery is proportional to the added GWAS' sample sizes and their r_g with melanoma, we screened potential

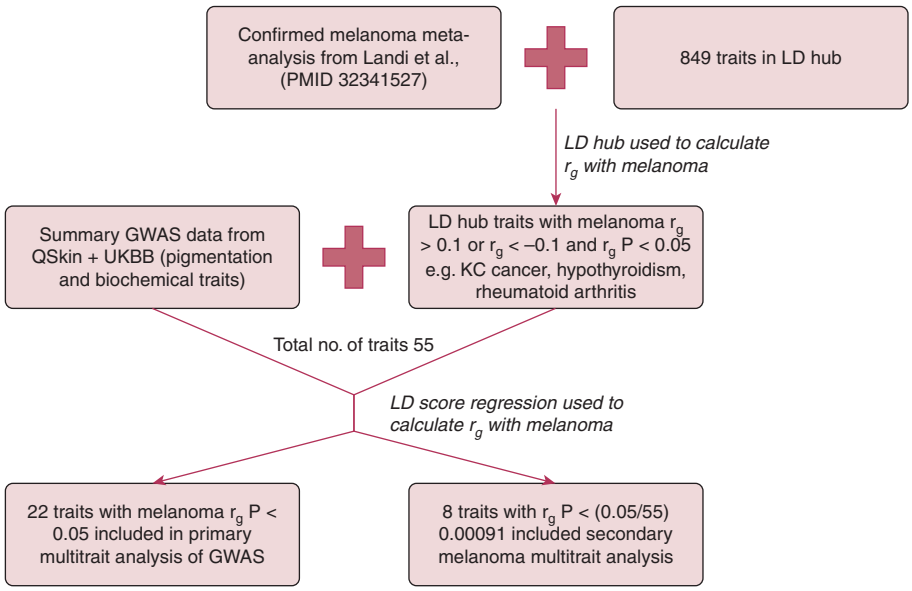

Figure 1. Study design. Flow chart of the study design. LD Hub was from Zheng et al. (2017); LD score regression was from Bulik-Sullivan et al. (2015a and 2015b). KC, keratinocyte; LD, linkage disequilibrium; r_g, genetic correlation; UKBB, United Kingdom Biobank; QSkin, QSkin Sun and Health Study.

traits using linkage disequilibrium (LD) score regression (Bulik-Sullivan et al., 2015a, 2015b). Following this selection process, we incorporated 22 GWAS of traits correlated with melanoma in MTAG to identify previously unreported SNPs associated with melanoma risk. As with all multi-trait approaches, there is the potential for false-positives where a SNP is not associated with melanoma but is associated (strongly) with another input trait; to validate our findings, we followed recommended best practices (Turley et al., 2019) and tested for replication of identified loci in an independent melanoma GWAS meta-analysis.

RESULTS

Genetic correlation between melanoma and other input traits

We assessed the correlation of melanoma, firstly, with a wide range of traits included in the LD Hub database and, secondly, with a selected set of traits of potential relevance to melanoma (many of which were not included in LD Hub, or were included only based on a limited sample size). We found traits associated with pigmentation were moderately correlated with melanoma, for example, skin color in QSkin $r_g \sim -0.6$ and KCs $r_g \sim 0.5$ (Table 1 and Supplementary Table S1). An exception to this was red hair; the low correlation with melanoma was likely a result of this not being a polygenic trait (Liu et al., 2018[1]). While the United Kingdom Biobank (UKBB) pigmentation r_g estimates were lower than those from QSkin, the confidence intervals overlapped, indicating comparable genetic architectures (Supplementary Table S2). As previously reported (Duffy et al., 2018), mole count and melanoma have a moderately strong $r_g = 0.57$ (standard error = 0.11). Interestingly, given the importance of the immune system in melanoma, we observed a correlation with autoimmune traits, including rheumatoid arthritis (RA) with r_g of −0.21 (standard error = 0.06); however, the exact biological relationship underlying this correction is not yet clear (Supplementary Table S2).

Multitrait analysis results

In a two-step process, we first prioritized a set of 55 traits where there was a correlation with melanoma before reducing them to a set of 22 traits (Figure 1, Methods). Our primary multitrait analysis included 22 traits we found to be genetically correlated with melanoma at an r_g $P < 0.05$ and to be having an effective sample size $N * r_g^2 > 400$ (Table 1 and Supplementary Table S2, Methods). The linkage disequilibrium score regression (LDSC) intercept for each included GWAS is in Supplementary Table S2.

In the multitrait results, we computed the LDSC intercept for the melanoma output trait from MTAG and found no inflation: 0.86 (standard error = 0.04) (Supplementary Table S2). Using FUMA (Methods [Watanabe et al., 2017]), we identified 74 independent genome-wide significant loci associated with melanoma (Supplementary Figure S1). A Q–Q plot is provided in Supplementary Figure S2. Of these loci, 19 have not previously reached genome-wide significance for melanoma (Table 2) (Landi et al., 2020).

As a sensitivity analysis, we performed a secondary analysis restricted to the eight traits where the r_g *P*-values were below the Bonferroni-corrected threshold of 0.001. As expected, due to the reduced effective sample size, fewer SNPs were genome-wide significant (60 SNPs) (Supplementary Table S3), but the concordance with the primary analysis results was high. A total of 51 of 60 SNPs from the secondary analysis were significant in the primary analysis, and effect sizes from the primary and secondary analyses were highly correlated ($r^2 = 0.89$) (Supplementary Figure S3).

Replication of MTAG melanoma results

To validate the loci identified by MTAG, we replicated our findings via two independent melanoma GWAS datasets

[1] Liu X, Loh PR, O'Connor LJ, Gazal S, Schoech A, Maier RM, et al. Quantification of genetic components of population differentiation in UK Biobank traits reveals signals of polygenic selection. bioRxiv 2018.

Table 2. New Loci Identified by a Multitrait Analysis of Melanoma

SNP	Chr	BP	EA/NEA	EA FREQ	Cutaneous Melanoma conf. *P*	Cutaneous Melanoma MTAG *P*	Cutaneous Melanoma MTAG OR (95% CI)	Replication *P*	Gene	Trait
rs1613999	**1**	**66,895,085**	**T/G**	**0.58**	**0.41**	**1.73×10^{-11}**	**1.06 (1.04–1.08)**	**4.95×10^{-4}**	***SGIP1***	***Pigm.***
rs10858023	**1**	**114,448,752**	**C/T**	**0.64**	**5.3×10^{-6}**	**2.18×10^{-10}**	**1.06 (1.04–1.08)**	**1.73×10^{-9}**	***AP4B1***	***AI***
rs231779	2	204,734,487	C/T	0.63	3.6×10^{-4}	2.22×10^{-11}	1.07 (1.05–1.09)	0.0381	*CTLA4*	*AI, KC cancer*
rs9846396	3	141,140,968	C/T	0.58	0.045	8.67×10^{-10}	0.95 (0.93–0.96)	0.0538	*RASA2*	*Pigm.*
rs9818780	3	156,492,758	T/C	0.53	2.1×10^{-6}	5.86×10^{-16}	0.93 (0.91–0.95)	7.13×10^{-4}	*LEKR1*	*Pigm.*
rs715199	**3**	**188,126,536**	**C/A**	**0.54**	**1.6×10^{-7}**	**3.68×10^{-9}**	**1.06 (1.04–1.07)**	**1.85×10^{-5}**	***LPP***	***AI***
rs11743151[1]	**5**	**38,756,717**	**C/T**	**0.66**	**1.6×10^{-6}**	**9.37×10^{-10}**	**1.06 (1.04–1.08)**	**1.32×10^{-11}**	***OSMR***	**—**
rs4714520	6	41,913,778	G/A	0.73	4.4×10^{-5}	1.30×10^{-10}	1.07 (1.05–1.09)	8.91×10^{-3}	*BYSL*	*Pigm., Meta.*
rs7761544	6	159,235,343	G/T	0.54	0.093	2.30×10^{-9}	0.95 (0.93–0.96)	0.0121	*EZR*	*Pigm.*
rs12350739	**9**	**16,885,017**	**G/A**	**0.47**	**0.44**	**8.24×10^{-67}**	**0.85 (0.84–0.87)**	**1.49×10^{-6}**	***BNC2***	***Pigm., KC cancer***
rs7098111	**10**	**119,573,178**	**C/T**	**0.84**	**7.6×10^{-4}**	**9.09×10^{-23}**	**1.13 (1.11–1.16)**	**7.12×10^{-8}**	***RAB11FIP2***	***Pigm.***
rs10899453	11	77,997,482	A/C	0.83	0.012	1.58×10^{-14}	1.1 (1.07–1.13)	7.09×10^{-3}	*USP35*	*Pigm.*
rs185790327[1]	12	88,996,942	G/A	0.92	0.24	5.47×10^{-9}	1.1 (1.06–1.13)	4.00×10^{-3}	*KITLG*	*Pigm.*
rs10774625	12	111,910,219	A/G	0.46	0.30	1.46×10^{-11}	0.94 (0.92–0.96)	0.1470	*SH2B3*	*AI, Meta.*
rs4470024	13	95,170,420	G/A	0.67	0.47	7.47×10^{-11}	0.94 (0.92–0.96)	0.2014	*DCT*	*Pigm., Meta.*
rs9923354	**16**	**4,450,421**	**T/G**	**0.24**	**1.1×10^{-4}**	**2.09×10^{-8}**	**0.94 (0.92–0.96)**	**1.97×10^{-6}**	***CORO7***	***Pigm.***
rs72833461	17	45,938,105	T/G	0.7	0.027	4.94×10^{-10}	0.94 (0.92–0.96)	0.1067	*SP6*	*Pigm.*
rs6565597	17	79,526,821	C/T	0.66	0.51	2.13×10^{-8}	0.95 (0.93–0.96)	0.9460	*FAAP100*	*Pigm.*
rs242132[1]	20	25,590,744	A/G	0.02	0.011	1.20×10^{-11}	1.22 (1.15–1.29)	0.0666[2]	*NANP*	*Pigm.*

Abbreviations: BR, base pair; Chr, chromosome; CI, confidence interval; conf., confirmed; EA, effect allele; EAF, effect allele frequency; KC, keratinocyte; Meta., metabolic; MIA, Melanoma Institute Australia; MTAG, multitrait analysis of GWAS; NEA, noneffect allele; Pigm., pigmentation.

Hg19 Chr, BP, EA and NEA, Haplotype Reference Consortium EAF, and nearest protein-coding gene (Gene) are reported. *P*-values for the input cutaneous melanoma clinically conf. meta-analysis (cutaneous melanoma conf. *P*) are provided, as are *P*-values, OR, and 95% CI for EA reported for the cutaneous melanoma MTAG of 23 GWAS datasets. We also report the MIA and 23andMe meta-analysis replication *P*-value; SNPs with replication *P*-values significant after Bonferroni correction ($P < 6.76 \times 10^{-4}$) are bolded (Methods). Full SNP results are in Supplementary Table S4. Trait indicates that the lead SNPs are associated at $P < 5 \times 10^{-8}$ with hair/skin color, sun burning, or tanning (Pigm.); rheumatoid arthritis or hypothyroidism (AI); KC; and metabolic trait (Meta). Details are provided in Supplementary Tables S7 and 8.

[1]Reported in Landi et al. (2020) but was not significant after multiple-testing correction.

[2]SNP not reported by 23andMe; only MIA results are reported.

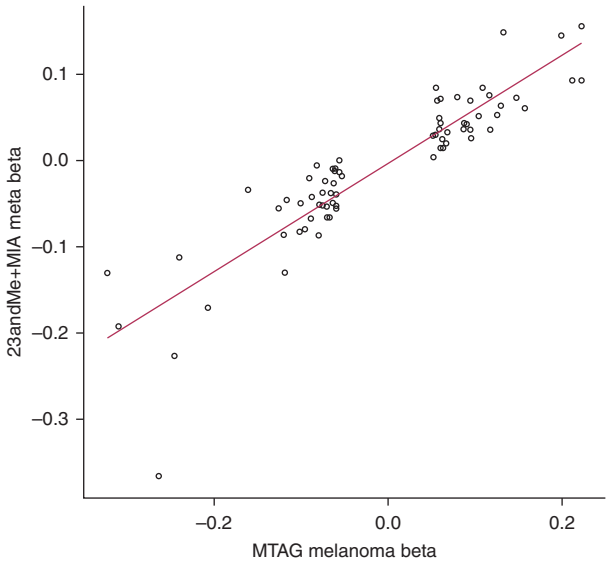

Figure 2. Correlation plot of replication sets and the MTAG melanoma output. For each lead SNP, we plot the corresponding log (OR) effect estimate from the MIA and 23andMe melanoma meta-analysis (Y-axis) against the effect size estimate from the MTAG of melanoma (X-axis). The coefficient of determination (r^2) = 0.81. MIA, Melanoma Institute Australia; MTAG, multitrait analysis of GWAS.

(Supplementary Table S4). The first was drawn from a cohort from Melanoma Institute Australia (MIA); there was no overlap with MIA samples used in the input melanoma GWAS meta-analysis (Landi et al., 2020). Manhattan plot and Q–Q plot of MIA set are provided in Supplementary Figures S4 and 5. The second replication set was from 23andMe, Inc. As expected, given the modest size of the MIA replication set relative to that of the large discovery MTAG, few SNPs were associated ($P < 0.05$) with melanoma in the MIA GWAS alone, with the exception of previously identified melanoma risk loci with moderately large effect sizes such as rs7943603 (*TYR*) ($P = 3.65 \times 10^{-7}$) (Supplementary Table S4). The majority of the previously reported melanoma SNPs were replicated in 23andMe, with exceptions of rs10782477 (*SLC24A4*), rs11217816 (*TMEM136*), rs72928038 (*BACH2*), and rs6424095 (*STPG1*). The previously reported melanoma GWAS loci reaching GWAS significance in the MIA replication set is provided in Supplementary Table S5.

To maximize our power, we meta-analyzed the MIA and 23andMe replication sets; 55 loci identified by MTAG were replicated at a Bonferroni-corrected *P*-value threshold of 6.76×10^{-4} (<0.05/74) (Supplementary Table S4), strongly suggesting that our multitrait approach has identified previously unreported loci associated with melanoma. Effect sizes from the multitrait analysis and the replication are highly correlated ($r^2 = 0.81$; Figure 2). In terms of the previously unreported loci discovered by MTAG, of the 19 loci not previously associated with melanoma, seven replicated at $P < 6.76 \times 10^{-4}$, including rs1613999 (*SGIP1*), rs10858023 (*AP4B1*), rs715199 (*LPP*), rs11743151 (*OSMR*), rs12350739 (*BNC2*), rs7098111 (*RAB11FIP2*), and rs9923354 (*CORO7*) (Table 1).

Comparison of *P*-values of significant SNPs across individual input GWAS

We compared lead melanoma MTAG SNPs with their significance across the individual input GWAS (Supplementary Table S7). The purpose of this comparison was to determine the likely functional relevance of these newly identified genetic variants. For example, rs231779 (*CTLA4*) was significantly associated with RA at $P = 1.20 \times 10^{-10}$ and hypothyroidism at $P = 8.71 \times 10^{-40}$ but not with pigmentation traits (UKBB skin color $P = 0.55$, UKBB child sunburn $P = 0.028$) (Okada et al., 2014; Zhou et al., 2018). With the caution that while the lead *CTLA4* SNP was associated with melanoma in the replication set ($P = 0.038$, Table 2), it did not reach the Bonferroni-corrected threshold of $P < 6.76 \times 10^{-4}$, this may suggest that the *CTLA4* locus is mediating melanoma risk through immune response rather than through traditional melanoma risk phenotypes. The *CTLA4* locus was also significantly associated with KC cancers (Liyanage et al., 2019), suggesting an overlap across skin cancers. Similarly, rs10858023 (*AP4B1*) has been associated with RA ($P = 5.00 \times 10^{-38}$) and hypothyroidism ($P = 2.06 \times 10^{-25}$), and rs715199 (*LPP*) has been associated with hypothyroidism ($P = 2.48 \times 10^{-16}$), but neither were associated with pigmentation ($P > 0.5$, Supplementary Table S7).

In contrast and as expected, given the importance of pigmentation in melanoma risk, many previously unreported loci such as rs1613999 (*SGIP1*) and rs12350739 (*BNC2*) were associated with skin and hair color, freckling, and skin burn type (Table 2 and Supplementary Table S7). Other traditional risk factors for melanoma are represented; rs11743151 (*OSMR*) is also associated with mole count ($P = 1.3 \times 10^{-4}$). Finally, we found evidence of potentially previously unreported pathways influencing melanoma risk. The SNP rs34517439 *(GIPC2)* was associated with multiple biochemical traits ($P = 3.5 \times 10^{-14}$) and alkaline phosphatase levels ($P = 1.4 \times 10^{-15}$) but was not significantly ($P < 5 \times 10^{-8}$) associated with pigmentation or autoimmune traits (Supplementary Table S7).

Annotation of associated loci

Following replication, previously unreported loci were explored for any associations with phenotypes other than melanoma, and expression quantitative trait loci datasets were interrogated to identify the likely candidate genes (Supplementary Materials and Methods). Putative drug targets were then identified on the basis of these candidate genes. This is summarized in Table 3; complete information is in Supplementary Table S8. Candidate genes at associated loci include several potential drug targets, including ones already used to treat melanoma (*CTLA4*) as well as those that are not reported previously to our knowledge. Results for gene-based analyses are reported in the Supplementary Note and Supplementary Table S6.

DISCUSSION

Using LD Hub and publicly available GWAS, we identified traits that exhibited r_g with melanoma (Supplementary Table S2). Building on this, we used a multitrait approach that leverages their r_g with melanoma and/or the large sample sizes of their respective GWASs, identifying 74 independent

Table 3. Annotation of Not Previously Reported Melanoma Loci

SNP	CHR	BP	eQTL Skin	eQTL Whole Blood	eQTL Other Tissue	PheWAS summary	Drug(s)	Usage
rs1613999	1	66,895,085	—	—	*SGIP1, TCTEX1D1*	Pigmentation, tanning	—	—
rs10858023	1	114,448,752	—	*AP4B1, BCL2L15, AP4B1-AS1*	*AP4B1, BCL2L15, PTPN22, RSBN1, AP4B1-AS1, MAGI3, DCLRE1B*	Auto(immunity)	—	—
rs231779	2	204,734,487	—	—	*CTLA4*	Auto(immunity)	*CTLA4* - IPILIMUMAB, TREMELIMUMAB - cancer inc.melanoma	*CTLA4* - IPILIMUMAB
rs9846396	3	141,140,968	*ZBTB38*	*ZBTB38*	*ZBTB38*	Anthropometric, pigmentation, cancer, auto(immunity)	—	—
rs9818780	3	156,492,758	*LINC00886, METTL15P1*	*LINC00886*	*LINC00886, METTL15P1, LEKR1, TIPARP*	Pigmentation, cancer	—	—
rs715199	3	188,126,536	—	—	—	Autoimmune	—	—
rs11743151	5	38,756,717	*LIFR-AS1*	—	—	—	—	—
rs4714520	6	41,913,778	—	*USP49*	*USP49, MED20, RP11-298J23.10*	Anthropometric	—	—
rs7761544	6	159,235,343	*EZR*	—	*EZR*	Pigmentation	—	—
rs12350739	9	16,885,017	—	*BNC2*	—	Pigmentation, tanning, BCC	—	—
rs7098111	10	119,573,178	—	—	—	—	—	—
rs10899453	11	77,997,482	*GAB2*	*NARS2*	*NARS2, USP35, GAB2, RP11-452H21.4, GAB2,*	Anthropometric, pigmentation, tanning, lymphocyte percentage	*NDUFC2* - METFORMIN - type II diabetes mellitus, polycystic ovary syndrome, SLE	—
rs185790327	12	88,996,942	—	—	—	—	—	—
rs10774625	12	111,910,219	*ALDH2*	—	*ALDH2, TMEM116, LINC01405*	Auto(immunity), anthropometric	*ALDH2*- DISULFIRAM alcohol dependence, infections, melanoma, cancer	*ALDH2* - DISULFIRAM - melanoma
rs4470024	13	95,170,420	—	—	—	—	—	—
rs9923354	16	4,450,421	*NMRAL1, CDIP1, HMOX2*	*NMRAL1, CDIP1*	*NMRAL1, CDIP1, CORO7, HMOX2, DNAJA3*	Pigmentation, tanning, anthropometric	—	—
rs72833461	17	45,938,105	—	—	—	Pigmentation, tanning platelet count	—	—
rs6565597	17	79,526,821	*TSPAN10*	*OXLD1, ARL16*	*TSPAN10, AC139530.1, ARL16*	Pigmentation, tanning anthropometric	*AC139530.1* - DIPYRIDAMOLE - stroke, diabetes mellitus, coronary artery disease, ischemia, rheumatoid arthritis, ovarian carcinoma, hypertension, HIV infection PENTOXIFYLLINE Hepatitis, alcohol intake, type II diabetes mellitus, chronic kidney disease, irritable bowel syndrome, infections, cancer	—

Abbreviations: BCC, basal cell carcinoma; BP, base pair; CHR, chromosome; eQTL, expression quantitative trait locus; WBC, white blood cell.

Hg19 CHR and BP positions are provided for each SNP. Only genes reaching the significance threshold of $5 \times 10^{-}$ or for PheWAS traits reaching $5 \times 10^{-}$ are shown. To limit the size of the table, we provide only a brief summary of the PheWAS traits; for full details of the identified traits, see Supplementary Table S8. For example, PheWAS results of ease of skin tanning, skin color, and childhood sunburn occasions have been summarized as pigmentation/tanning. The usage field indicates whether the listed drug is currently used or undergoing clinical trials for melanoma. Auto(immunity) indicates PheWAS traits, including both general immune traits (e.g., WBC levels) and autoimmune disorders. Autoimmune refers solely to autoimmune traits.

genome-wide significant loci (Supplementary Table S8); these comprise those previously identified for melanoma risk variants as well as previously unreported variants (Table 2). Of particular interest is rs231779 (MTAG melanoma $P = 2.22 \times 10^{-11}$; OR = 1.07, 95% CI 1.05−1.09), which is an expression quantitative trait locus (eQTL) for the *CTLA4* gene. CTLA4 is targeted by ipilimumab for melanoma treatment (Gensous et al., 2018), and although not a previously reported clinical target, this provides proof of principle that a multitrait approach can identify genes that are drug targets. However, the result of rs231779 must be viewed with caution because it did not reach the Bonferroni-corrected *P*-value threshold (cutaneous melanoma association $P = 0.038$).

Findings from multi-trait GWAS analyses can be difficult to interpret, given that the inflation in MTAG test statistics is sensitive to the input traits and can potentially generate false-positive findings (Turley et al., 2019). We used two approaches to address this concern. First, we conducted a secondary multi-trait analysis limited to the eight traits with a melanoma r_g $P < 0.00091$ (Table 1). SNP effect sizes were highly concordant between the primary and secondary analyses ($r^2 = 0.89$, Supplementary Figure S3), and the majority (51 of 60) of the loci significant in the secondary analysis were also identified in the primary analysis (Supplementary Table S3). Second, we required replication in an independent melanoma GWAS. Together, these checks give us confidence that our multitrait approach is overall identifying loci associated with melanoma.

As expected, many of the newly identified SNPs were associated with pigmentation (Table 2 and Supplementary Table S8). However, we also identified the SNPs associated with other pathways. rs715199 has been associated with hypothyroidism and KC (Liyanage et al., 2019) but not with any of the pigmentation traits in our analysis (Supplementary Table S7). This SNP is in LD r^2 with rs13076312, which has been associated with vitiligo (Shen et al., 2016). rs715199 was an eQTL for *BCL6*, which is involved in the development of T-follicular cells; these cells are implicated in skin inflammation and autoimmune disorders (Gensous et al., 2018; Sabat et al., 2019). This may highlight further evidence of a shared pathway between autoimmune conditions and skin cancer.

Our multitrait analysis included RA, which is treated with immunomodulatory biologics that have been associated with a modestly increased risk for melanoma, OR of 1.20–1.56 depending on the specific agent (de Germay et al., 2020; Esse et al., 2020). If treatment-induced melanoma is common, this misclassification or misdiagnosis could potentially lead to (spurious) genetic correlation. In the absence of a true genetic correlation, one-way misdiagnosis where disease A is misdiagnosed as disease B proportionally generates a spurious r_g (Wray et al., 2012). However, given these modest ORs and the low prevalence of both RA (∼1−2%) and cutaneous melanoma (∼2.5% depending on population and latitude), the proportion of RA cases misdiagnosed as cutaneous melanoma owing to medication effects and resultant cryptic positive r_g would be very low (<2%). As we find a negative correlation between RA and melanoma (Table 1), it is unlikely that treatments are driving the observed genetic correlation. We also observed a genetic correlation with hypothyroidism; because this disease is not treated by immunomodulatory biologics, it further supports a genetic overlap between melanoma and autoimmunity.

In addition to a standard GWAS meta-analysis, Landi et al. (2020) identified potential additional melanoma risk loci by performing *P*-value meta-analyses with mole count, or hair color GWAS and also through transcriptome-wide association approaches (Landi et al., 2020) (Supplementary Table S8). Of these, rs34517439 (*GIPC2*), rs2453042 (*ADAM30*), rs10948654 (*AL355997.1*), rs9322309 (*AKAP12*), rs10498512 (*SYNE2*), and rs11625064 (*ZFP36L1*) had $P < 0.05$ in the combined replication sets, suggesting that they may be real melanoma risk loci (Supplementary Table S4).

Strengths and limitations

Using MTAG, which models the r_g (estimated using LD score regression) between input traits, we have combined a clinically confirmed melanoma GWAS with genetically correlated traits, greatly improving our power to detect previously unreported loci. Each contributing GWAS utilized an ethnically homogeneous European ancestry population and controlled for residual population stratification, reducing the possibility of spurious associations due to population substructure. To validate our findings, we drew on two large, independent melanoma GWAS, with 55 of 74 melanoma MTAG loci associations showing strong ($P < 6.76 \times 10^{-4}$) evidence of being replicated. We identified further pigmentation loci as well as loci potentially related to the immune system, enhancing our understanding of genetic pathways to melanoma.

However, some GWAS include self-reported data or cases where histological confirmation was performed for only a fraction of study participants. We have previously found that 23andMe's self-report melanoma phenotype used in the replication set has $r_g \sim 1$ with clinically confirmed melanoma (Landi et al., 2020). The KC cancers data used in this study from the QSkin included cases identified through Australian Medicare data. While these records are highly reliable in terms of identifying KC cancers (Thompson et al., 2016) because pathology records were only available for a subset of QSkin KC cancers cases, they could not be assigned a specific diagnosis of either BCC or SCC (Liyanage et al., 2019). UKBB BCC and SCC data include self-reports that are confirmed with the national cancer registers (Sudlow et al., 2015). However, the r_g with melanoma (Supplementary Table S2) were similar for both UKBB KC cancers and QSkin KC cancers (r_g of 0.48 vs. 0.46), suggesting that they represent comparable phenotypes.

Conclusion

We undertook a large-scale multitrait analysis of cutaneous melanoma and correlated traits. We identified 19 loci not previously associated with melanoma, of which seven replicated after correction for multiple testing. The pathway of greatest interest identified in this study relates to autoimmunity; the key previously unreported discoveries include rs715199 (*LPP*) and rs10774625 (*SH2B3*). Treatments targeting *CTLA4* have contributed to a transformation in the management of advanced melanoma, and identification of further genes involved in both autoimmunity and melanoma

is promising. Future functional analyses of the loci reported in this study to confirm the underlying genes may identify not previously reported targets for chemoprevention of melanoma.

MATERIALS AND METHODS

Identifying traits correlated with melanoma for a multitrait analysis

We performed a stepwise approach (Figure 1) using bivariate linkage disequilibrium (LD) score regression (LDSC) (version 1.0.0) to identify traits genetically correlated with a large clinically confirmed cutaneous melanoma GWAS meta-analysis (see the following paragraphs) (Supplementary Table S9) (Landi et al., 2020). LD score is a measure of the number of additional SNPs in LD with a given genetic variant and can be calculated from an appropriate reference set enabling its application to GWAS summary data (Bulik-Sullivan et al., 2015a, 2015b). Because a given SNP's association with melanoma or its bivariate association with two traits is influenced by all other genetic variants it is in LD with, r_g can be estimated by the regression of the association of a given SNP with two traits on its LD score (Bulik-Sullivan et al., 2015, 2015b).

In the first step, we used LD Hub, which implements bivariate LDSC, to screen its database of 849 GWAS (Bulik-Sullivan et al., 2015a; Zheng et al., 2017). The purpose of this stage was to identify traits or GWAS genetically correlated with melanoma that could be included in a multitrait analysis. We considered all immune traits entered in the LD Hub, including ulcerative colitis, systemic lupus erythematosus, and Crohn's disease; however, none of these additions were strongly correlated with melanoma. The results of this screen are reported in Supplementary Table S1. GWAS or traits with a melanoma $r_g > 0.1$ or < -0.1 and with a $P < 0.05$ were identified as suitable for inclusion in stage 2.

As the second step, where available, summary GWAS results were downloaded for the selected stage 1 traits. Where suitable phenotypes were available for the selected stage 1 traits, GWASs were performed directly in UKBB and QSkin cohorts. The initial list of traits derived from stage 1 was supplemented by additional GWASs that were not included in LD Hub. Added traits included GWAS known to be associated with melanoma risk from previous studies but not themselves included in LD Hub (e.g., GWAS of pigmentation, BCC, and SCC [Liyanage et al., 2019] or mole count [Duffy et al., 2018]). We also considered GWAS with large sample sets but without previous evidence of association (e.g., risky behavior, total bilirubin). Together, stage 2 comprised data from UKBB, QSkin, and dbGaP, including metabolic measurements. Specific details describing the contributing cohorts, data cleaning, and analysis of specific trait GWAS within these cohorts are in the Supplementary Note.

Not all traits identified through LD Hub were included in the second step, either owing to redundancy (e.g., number of self-reported cancers from the UKBB includes both melanoma and KC cancers, GWAS of which were included in the MTAG directly) or because we could not access the necessary GWAS summary data such as for lung cancer.

The r_g with melanoma for these GWAS was then confirmed with bivariate LDSC. In total, 55 GWASs were surveyed in this second step (Supplementary Table S2).

The sample size required to detect association on the basis of a correlated variable (e.g., between a marker SNP and an unmeasured causal SNP) is $\sim 1/r^2$ (Pritchard and Przeworski, 2001); extending this concept, the effective gain in sample size in a multitrait genetic analysis is proportional to r_g^2. To ensure that we are only including well-powered GWAS of relevance to melanoma, we further filtered the 55 GWAS (Supplementary Table S2) to those with a melanoma r_g $P < 0.05$ and a proportional multitrait sample size ($N \times r_g^2$) > 400 (selected arbitrarily; as a guide, 400 cases is $\sim$0.5% of the effective sample size of the input melanoma GWAS meta-analysis), where N is the total sample size for quantitative GWAS, or 4/(1/case + 1/controls) for case-control GWAS.

This left 22 GWASs, which we used in the primary multitrait analysis (Table 1). Weighting traits by their r_g with melanoma allows for straightforward inclusion of GWAS where the phenotype coding is reversed; for example, skin burn type in QSkin and UKBB is coded in terms of greater and less burning, respectively (Table 1). Genome-wide significance for the multitrait analysis of melanoma was set to $P < 5 \times 10^{-8}$.

As a secondary sensitivity analysis to ensure that including GWASs only modestly correlated with melanoma was not strongly affecting our results, we further filtered input GWAS to those with a melanoma r_g $P < 0.00091$ (0.05 corrected for the 55 GWAS). This left eight GWASs: KC cancers (UKBB), skin burn type (QSkin), mole count (Duffy et al., 2018), potassium in urine (UKBB), skin color (QSkin), RA (Okada et al., 2014), KC cancers (QSkin), and skin tan type (QSkin) (Table 1). Effect sizes for lead SNPs identified in the primary analysis were compared with those from the secondary analysis (Supplementary Figure S3).

Study participants

For this analysis, we had access to summary data from a recent clinically confirmed melanoma GWAS meta-analysis (Landi et al., 2020): the QSkin cohort (Olsen et al., 2012) and the UKBB (Sudlow et al., 2015). A detailed description of each cohort and additional publicly available GWAS datasets is provided in Supplementary Table S2 and the Supplementary Note. All study participants were from European ancestry populations. Written informed consent has been obtained from each participant in each individual study (Landi et al., 2020; Olsen et al., 2012; Sudlow et al., 2015).

Melanoma replication sets

To confirm whether the loci identified by MTAG were associated with melanoma risk, we used two independent replication sets from MIA and 23andMe, Inc. A full description of each dataset and methods used in meta-analysis of the replication datasets is in Supplementary Note. Results for each of the 74 lead SNPs from the melanoma MTAG loci were combined using a fixed-effects inverse-variance weighted meta-analysis (Table 2 and Supplementary Table S4). The SNP effect sizes for the MTAG output versus the replication meta-analysis set are plotted in Figure 2.

Loci identification, gene-based analysis, and annotation of GWAS loci

Identifying independent lead SNP and loci was performed in the functional mapping and annotation application (Watanabe et al., 2017). Gene-based analysis was used to identify additional loci that were not significant in the single SNP MTAG using FastBAT (Bakshi et al., 2016). Post-GWAS annotation, including MAGMA gene-set enrichment analysis, was conducted in functional mapping and annotation (Watanabe et al., 2017). For full details, see the Supplementary Note.

Data availability statement

The genome-wide summary statistics from 23andMe data were obtained under a data transfer agreement. Further information about obtaining access to the 23andMe summary statistics is available from https://research.23andme.com/collaborate/. The raw genetic and phenotypic UK Biobank data used in this study, which were used under license, are available from http://www.ukbiobank.ac.uk/. For nevus data, see the study by Duffy et al. (2018). For QSkin data, see the study by Olsen et al. (2012). For melanoma GWAS meta-analysis, see the study by Landi et al. (2020). For rheumatoid arthritis GWAS summary data, see the study by Okada et al. (2014), for hypothyroidism, see the study by Zhou et al. (2018), and for risky behavior data, see the study by Karlsson Linnér et al. (2019). Keratinocyte cancer data (eMERGE) are available through dbGaP (dbGaP identification phs000360) and Kho et al. (2011).

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CONFLICT OF INTEREST

JFT has received honoraria for advisory board participation from BMS Australia, MSD Australia, GSK, and Provectus and travel support from GlaxoSmithKline and Provectus. RPMS has received honoraria for advisory board participation from MSD Australia, Novartis, and QBiotics and speaking honoraria from BMS Australia and Novartis. GVL is a consultant advisor to Aduro Biotech, Amgen, Array Biopharma, Boehringer Ingelheim International GmbH, Bristol-Myers Squibb, Highlight Therapeutics S.L., Merck Sharp & Dohme Australia, Novartis Pharma AG, QBiotics Group, Regeneron Pharmaceuticals, and SkylineDX B.V. Specialised Therapeutics Australia Pty. RAS has received fees for professional services from QBiotics, Novartis, Merck Sharp & Dohme, NeraCare, AMGEN, Bristol-Myers Squibb, Myriad Genetics, and GlaxoSmithKline. YJ and CT are employed by and hold stock or stock options in 23andMe. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS

DCW was funded by a fellowship from the National Health and Medical Research Council of Australia APP1155413. MMI acknowledges funding from Cancer Research UK (project grant C8216/A6129 and program award C588/A4994) and from the National Institutes of Health (Bethesda, MD) (R01 CA83115). MTL acknowledges funding from the Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health. GVL and RAS are supported by National Health and Medical Research Council Program Grant and Practitioner Fellowships. GVL is supported by the University of Sydney Medical Foundation. RPMS is supported by Melanoma Institute Australia. The Australian Genetics of Depression Study was primarily funded by the National Health and Medical Research Council of Australia grant 1086683 and was further supported by National Health and Medical Research Council grants 1145645, 1078901, and 1087889.

AUTHOR CONTRIBUTIONS

Conceptualization: SM, MHL, UEL; Data Curation: YJ, SGG, YJ, CT, UEL, RAS, GVL, JFT, AS, RPMS, JSt, JSh, MTL, SEM, EMB, NGM; Formal Analysis: UEL, MHL, YJ, CT, SGG, DLD, DTB, MMI, MTL, JSh, JA, JSO, XH; Funding Acquisition: MHL, SM, MTL, CMO, DCW, SEM, EMB, NGM, RAS, GVL; Investigation: SM, MHL, UEL, RAS, GVL, JFT, AS, RPMS, JSt, JSh, MTL, CMO, DCW, SEM, EMB, NGM, ACG, DLD, DTB, MMI, JA, JSO, XH; Methodology: SM, MHL, UEL; Project Administration: MHL, SM; Resources: SM, SGG, YJ, CT, MHL, RAS, GVL, JFT, AS, RPMS, JSt, JSh, MTL, CMO, DCW, SEM, EMB, NGM, DLD, ACG, DTB, MMI, JA, JSO, XH; Software: MHL, SGG, UEL, XH; Supervision: SM, MHL; Validation: MHL, SM, UEL, YJ, CT, RAS, GVL, JFT, AS, RPMS, JSt, JSh, MTL, SEM, EMB, NGM; Visualization: UEL, MHL; Writing - Original Draft Preparation: UEL, MHL, SM; Writing - Review and Editing: UEL, SM, DTB, JSh, JA, JSO, XH, RAS, NGM, SEM, EMB, ACG, RPMS, JFT, JSt, AS, YJ, CT, SGG, DLD, CMO, DCW, GVL, MMI, MTL, MHL

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.08.449.

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