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Article

# Different Pigmentation Risk Loci for High-Risk Monosomy 3 and Low-Risk Disomy 3 Uveal Melanomas

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## Abstract

**Background:** Uveal melanoma (UM), a rare malignant tumor of the eye, is predominantly observed in populations of European ancestry. UMs carrying a monosomy 3 (M3) frequently relapse mainly in the liver, whereas UMs with disomy 3 (D3) are associated with more favorable outcome. Here, we explored the UM genetic predisposition factors in a large genome-wide association study (GWAS) of 1142 European UM patients and 882 healthy controls . **Methods:** We combined 2 independent datasets (Global Screening Array) with the dataset described in a previously published GWAS in UM (Omni5 array), which were imputed separately and subsequently merged. Patients were stratified according to their chromosome 3 status, and identified UM risk loci were tested for differential association with M3 or D3 subgroups. All statistical tests were 2-sided. **Results:** We recapitulated the previously identified risk locus on chromosome 5 on *CLPTM1L* (rs421284: odds ratio [OR] =1.58, 95% confidence interval [CI] = 1.35 to 1.86; $P = 1.98 \times 10^{-8}$) and identified 2 additional risk loci involved in eye pigmentation: *IRF4* locus on chromosome 6 (rs12203592: OR = 1.76, 95% CI = 1.44 to 2.16; $P = 3.55 \times 10^{-8}$) and *HERC2* locus on chromosome 15 (rs12913832: OR= 0.57, 95% CI = 0.48 to 0.67; $P = 1.88 \times 10^{-11}$). The *IRF4* rs12203592 single-nucleotide polymorphism was found to be exclusively associated with risk for the D3 UM subtype ($OR_{D3} = 2.73$, 95% CI = 1.87 to 3.97; $P = 1.78 \times 10^{-7}$), and the *HERC2* rs12913832 single-nucleotide polymorphism was exclusively associated with risk for the M3 UM subtype ($OR_{M3} = 2.43$, 95% CI = 1.79 to 3.29; $P = 1.13 \times 10^{-8}$). However, the *CLPTM1L* risk locus was equally statistically significant in both subgroups. **Conclusions:** This work identified 2 additional UM risk loci known for their role in pigmentation. Importantly, we demonstrate that UM tumor biology and metastatic potential are influenced by patients' genetic backgrounds.

Uveal melanoma (UM) arises from melanocytes in the uveal tract of the eye, including the choroid and, more rarely, ciliary body and iris. Prognosis is dismal when the disease spreads, frequently metastasizing to the liver (1). Loss of chromosome 3 and gain of chromosome 8 are associated with a higher risk of metastatic relapse (2,3). Monosomy 3 (M3) UMs are associated with *BAP1* (3p21) mutations and a high risk of metastases (4). Conversely, disomy 3 (D3) tumors carry *SF3B1* or *EIF1AX* mutations (5-7) and are associated with late metastases and a better prognosis. These M3 and D3 subtypes are different not only in terms of mutational statuses but also at the cytogenetic, miRNome, methylome, and proteome levels, suggesting that they derive from 2 tumorigenic processes (8).

UM mainly affects populations of European ancestry, with a 10-fold lower incidence in individuals of African American or

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Asian Pacific Islander ancestry (9,10). Fair skin and blue–gray eyes are also risk factors for UM (11). With the hypothesis that higher frequency of risk alleles exists in populations of European ancestry to explain UM epidemiology, we performed the first genome-wide association study (GWAS) in UM and identified rs421284 as the leading single-nucleotide polymorphism (SNP) on the *CLPTM1L/TERT* risk locus on chromosome 5p15.33. Moreover, a trend for association between variants in *OCA2* and UM was also observed (12). Recently, another UM GWAS identified 11 loci with a *P* value of association less than $10^{-5}$, but none reached statistical significance (13).

The *CLPTM1L* risk allele identified by our first UM GWAS had a higher frequency in individuals of African American ancestry compared with Europeans and thus could not explain the peculiar prevalence of UM in individuals of European ancestry (12). To identify additional UM risk loci in the European population, we increased the power of our GWAS by performing genome-wide genetic imputation and by accruing 1142 UM patients and 882 controls, a threefold increase of our first study, allowing subgroup analysis depending on chromosome 3 status.

## Methods

### Study Populations

This study was approved by the ethical committee and internal review board at the Institut Curie. Blood samples were obtained from 946 UM patients who consented to participate in the study and from 496 control individuals of French origin from the KIDRISK consortium (US NCI U01CA155309; G. Scelo). Genotypes obtained on the Infinium Global Screening Array 24 v1.0 were called using default parameters in GenomeStudio (Illumina).

### Genotyping, Imputation, and Merge

Genotypes from the previously published GWAS (dataset1) (12) and for the 2 new sets (dataset2 and dataset3) were filtered (Supplementary Methods, available online) and independently imputed on the Michigan Imputation Server using Eagle for the phasing and Haplotype Reference Consortium r1.1 as the reference dataset. Imputed datasets were merged together, and another quality control was performed (Supplementary Table 1, available online). Manual genotyping was also performed on selected SNPs and individuals (Supplementary Methods, available online). Patients and controls of European ancestry were stringently selected for further analyses (Supplementary Methods and Supplementary Figures 1 and 2, available online).

### Statistical Analysis

For GWAS, firth logistic regression was performed using plink2 with covariates described in the Supplementary Methods (available online). An exact number of patients and controls used are indicated in the respective figures and tables for each analysis. Association of SNPs with UM risk was determined by odds ratios (ORs) with 95% confidence intervals (CIs), and SNPs with a *P* value less than $5.00 \times 10^{-8}$ were considered to be statistically significant, and those with *P* value less than $1.00 \times 10^{-5}$ only reached the tendency line. Eye color was predicted using IrisPlex tools (https://hirisplex.erasmusmc.nl/). Association of eye color with UM risk was calculated using a 2-sided Fisher test *P* value and odds ratio. Comparison of variant allele frequency (VAF) of SNPs in different populations were tested for statistical significance using a 2-sided Fisher test *P* value. Expression quantitative trait loci (eQTL) were performed using linear regression. A *P* value of less than .05 was considered statistically significant for all tests other than GWAS firth logistic regression.

## Results

### Genome-Wide Association Study in UM

We combined 2 independent datasets (dataset2: 369 UM and 496 controls; dataset3: 577 UM, Global Screening Array) with that of our previous UM GWAS (dataset1 of 271 UM and 429 controls; Omni5 array) (12). The data were quality filtered (Figure 1; Supplementary Table 1, available online). The 3 datasets were imputed separately using the Haplotype Reference Consortium on the Michigan server and subsequently merged. Quality of the genotyping and imputation was further assessed by TaqMan genotyping on rs421284, rs12203592, and rs12913832 SNPs on 972 selected samples, with 95.2%, 99.1%, and 99.6% of good match, respectively (Supplementary Table 2, available online). Data from individuals of European ancestry were stringently selected from principal component analyses (PCA) using plink2 in which the first 2 principal components were used. Outliers were then excluded from those selected samples using SmartPCA with 10 iterative PCAs (Supplementary Figures 1-3, available online). The final dataset for the UM GWAS analysis consisted of 7 488 175 SNPs in 1142 patients and 882 controls (Figure 1).

The GWAS Manhattan plot showed 3 distinct loci reaching genome-wide significance (firth logistic regression $P < 5.00 \times 10^{-8}$) (chr5, *CLPTM1L/TERT* locus; chr6, *IRF4* locus; and chr15, *HERC2/OCA2* locus) (Figure 2; Supplementary Table 3, available online). Within the *HERC2/OCA2* locus, 8 SNPs in high linkage disequilibrium reached statistical significance. The most statistically significant SNPs at this locus were rs1129038 and rs12913832 (OR = 0.56, 95% CI = 0.48 to 0.66; $P = 5.97 \times 10^{-12}$; and OR = 0.57, 95% CI = 0.48 to 0.67; $P = 1.88 \times 10^{-11}$, respectively), located in *HERC2*. A single SNP located in *IRF4* was found to be well above the genome-wide significance: rs12203592 (OR = 1.76, 95% CI= 1.44 to 2.16; $P = 3.55 \times 10^{-8}$). Finally, the association study recapitulated the previously identified 5p15.33 risk locus (*TERT/CLPTM1L*) (12), with several SNPs in high linkage disequilibrium ($r^2 > 0.9$) reaching statistical significance (Supplementary Table 3, available online). The most statistically significant SNP was rs370348 (OR = 1.59, 95% CI = 1.35 to 1.86; $P = 1.48 \times 10^{-8}$)., The leading risk SNP in our first GWAS, rs421284 (12), also showed high statistical significance (OR = 1.58, 95% CI = 1.35 to 1.86; $P = 1.98 \times 10^{-8}$) and was further analyzed in this study. A few other loci showed suggestive evidence for an association with UM but did not reach genome-wide significance ($P < 5.00 \times 10^{-8}$) (Supplementary Table 3, available online and Figure 2).

Conditional analyses enable the detection of secondary independent association signals within a genomic locus by conditioning on the primary associated SNP at the locus. At the *CLPTM1L*, *IRF4*, and *HERC2* loci, no other statistically significant SNP was found to be independently associated with UM when conditioning on rs421284, rs12203592, or rs12913832, respectively. Moreover, these 3 conditional analyses did not reveal any statistically significant regions other than *CLPTM1L*, *IRF4*, and *HERC2* (Supplementary Figure 4, available online).

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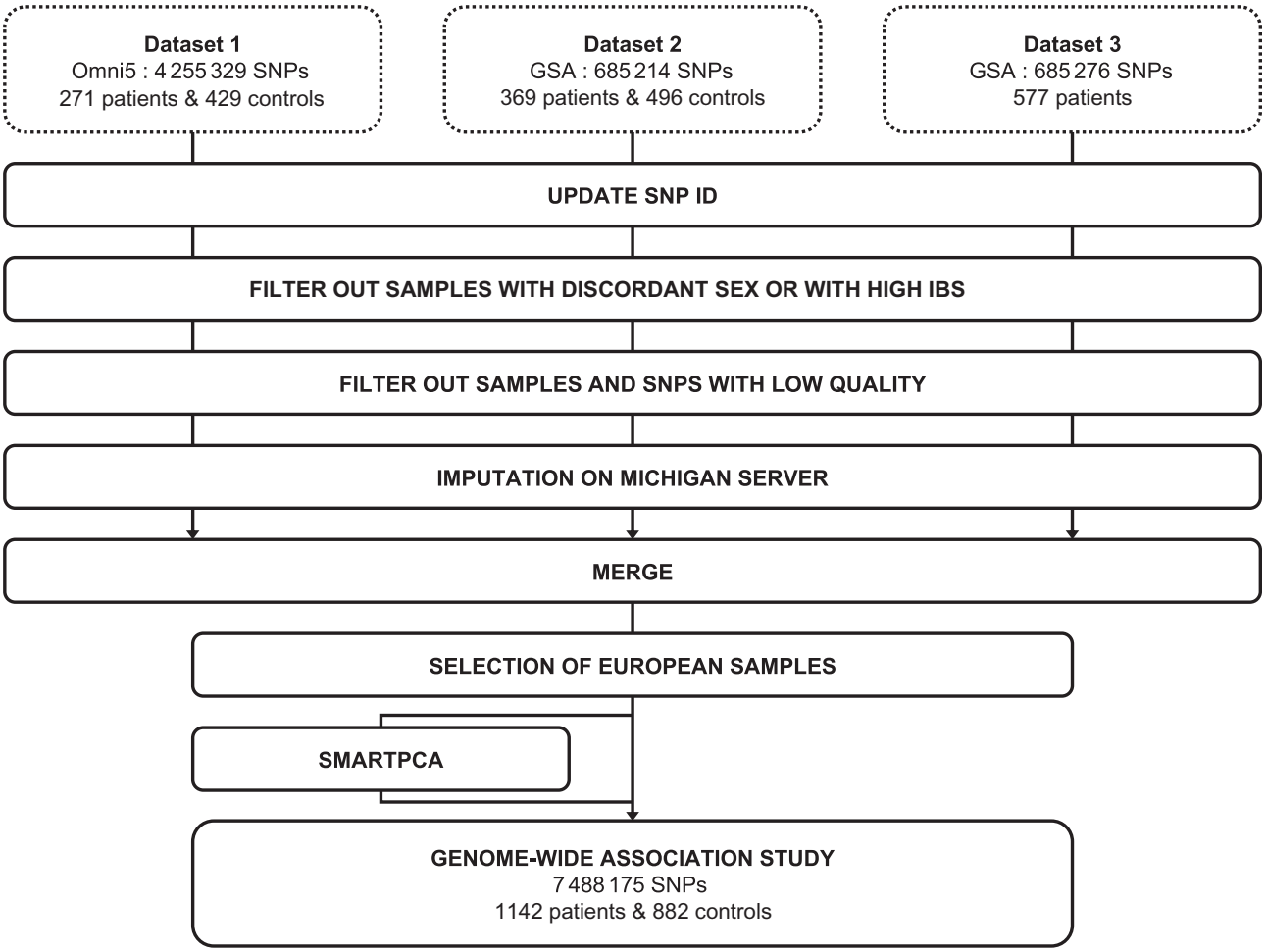


**Figure 1.** Files and pipeline used for the filtering and imputation of the Genome-Wide Association Study in uveal melanoma. GSA = Global Screening Array; ID = identification; SNP = single nucleotide polymorphism; IBS = Identify By State.

## UM Risk Loci and Pigmentation

To evaluate the impact of risk SNPs on gene regulation, eQTL analyses were performed for the statistically significant loci using expression data from tumors of an in-house series of 73 UMs (14). We previously identified an association between *CLPTM1L* expression and rs421284 with higher expression of *CLPTM1L* in individuals carrying the risk allele (C) (12). Interestingly, the other 2 major risk loci identified in this association study, *IRF4* and *HERC2*, are known to be strongly implicated in the regulation of the pigmentation pathways determining eye and skin colors (15-17), prompting us to further investigate the expression of pigmentation genes in UM. *IRF4* expression was found to be strongly associated with rs12203592 alleles, with a decreased expression in tumors carrying the risk TT genotype (linear regression $P = 2.00 \times 10^{-6}$; Supplementary Figure 5, A, available online). Looking at eQTLs in the Genotype-Tissue Expression database, rs12203592 is linked to *IRF4* expression in most tissues, but the directionality of the association varies. As in UM, sun-exposed skin had a lower *IRF4* expression linked to the T allele, whereas a lower expression of *IRF4* is associated with the C allele in all other tissues, suggesting a tissue-specific regulation for this gene (Supplementary Figure 5, B, available online). At the *HERC2* locus, no correlation was found between rs12913832 alleles and expression of this gene in UM (Supplementary Figure 6, A, available online), in contrast to whole blood, where there is a statistically significant decrease in *HERC2* expression associated with the G allele (Supplementary Figure 6, B, available online). However, expression of *OCA2*, a nearby gene known to be regulated by HERC2 in melanocytes (17), was found with a highly statistically significant association with rs12913832 genotypes ($P = 9.08 \times 10^{-4}$) in UM, with decreased expression for tumors carrying the risk G allele (Supplementary Figure 6, C, available online).

Our finding of 2 major pigmentation loci is in accordance with the high prevalence of light eye color in UM patients of European ancestry (11). We investigated whether the risk of developing UM conferred by the risk alleles of *HERC2* and *IRF4* was fully linked to their determining role in eye pigmentation. We thus predicted the eye color of all UM and control individuals included in this study, using the algorithm developed in the IrisPlex System, based on the genotype combination of 6 SNPs (*HERC2* rs12913832, *OCA2* rs1800407, *SLC45A2* rs16891982, *TYR* rs1393350, *IRF4* rs12203592, and *LOC105370627*: intron variant) (18). We predicted the eye color of UM patients and controls to be brown (41.6% of patients vs 60.1% of controls, respectively), green (1.7% vs 1.1%), or blue (56.7% vs 38.9%), allowing us to confirm the statistically significant association of blue eye color (vs other eye colors) with UM risk (OR = 2.07, 95% CI = 1.72 to 2.49; 2-sided Fisher test $P = 1.21 \times 10^{-15}$) (Figure 3, A and B), confirming the recent study by Jager and colleagues (19). Strikingly, when we added eye color

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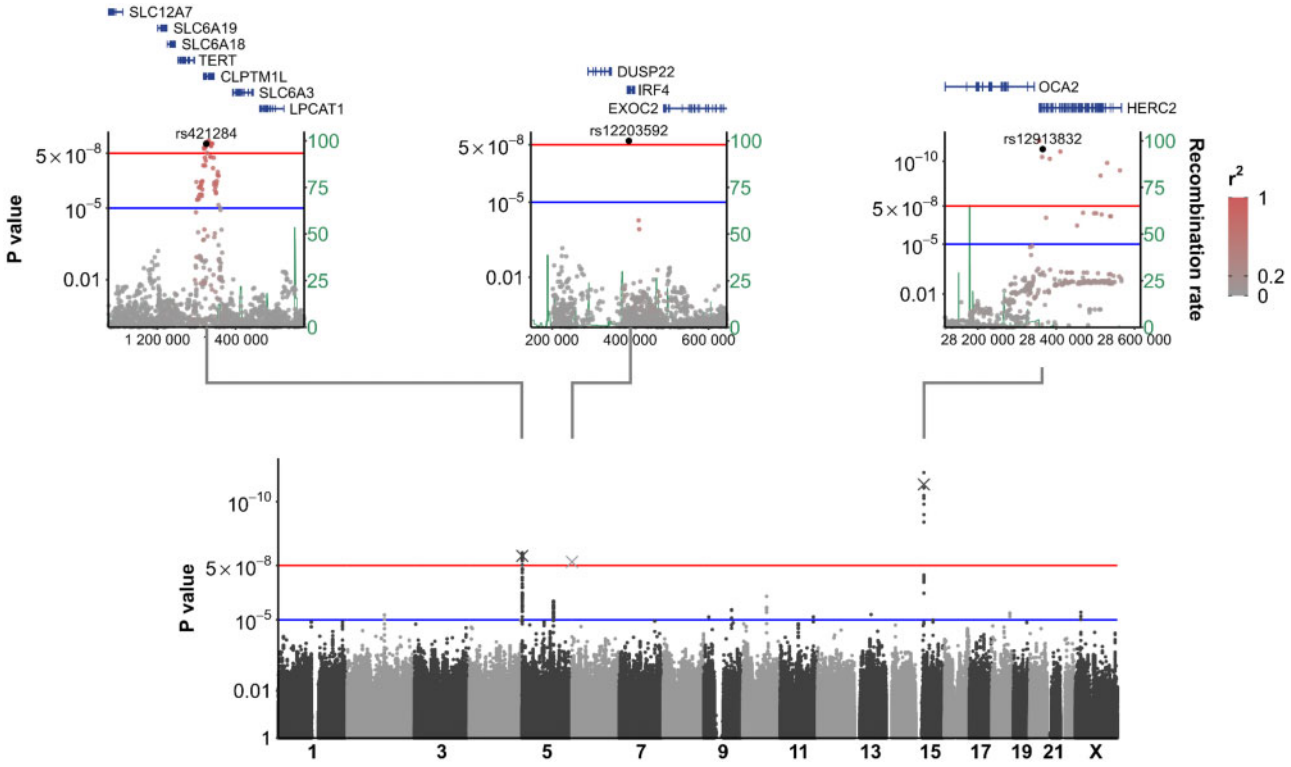


**Figure 2.** Manhattan plot and regional linkage disequilibrium plot for statistically significant loci. For the Manhattan plot, the association test *P* value (**y-axis**) is plotted against its physical chromosomal position (**x-axis**). Chromosomes are shown in alternating black and grey. SNPs above the **top horizontal line** represent those with a $P < 5.00 \times 10^{-8}$ and were considered to be statistically significantly associated with uveal melanoma. The **bottom horizontal line** represents the tendency line ($P < 1.00 \times 10^{-5}$). Statistical significance was measured using unconditional logistic regressions. For regional locus plots, genes are depicted with **rectangles** and SNPs are represented by **dots**. Shading of dots reflects the level of linkage disequilibrium ($r^2$) with the highlighted SNP of interest (**black circle** with rs number indicated). **Vertical bars** indicate recombination rates in human population. *CLPTM1L* = cleft lip and palate transmembrane protein 1-like; *DUSP22* = dual specificity phosphatase 22; *EXOC2* = exocyst complex component 2; *HERC2* = HECT and RLD domain containing E3 ubiquitin protein ligase 2; *IRF4* = interferon regulatory factor 4; *LPCAT1* = lysophosphatidylcholine acyltransferase 1; *SLC12A7* = solute carrier family 12 member 7; *SCL6A18* = solute carrier family 6 member 18; *SCL6A19* = solute carrier family 6 member 19; *SCL6A3* = solute carrier family 6 member 3; *OCA2* = oculocutaneous albinism II; *TERT* = telomerase reverse transcriptase.

(determined by the IrisPlex System) as a covariate in the association analysis, the resulting odds ratio remained unchanged for *IRF4* (OR = 1.76, 95% CI = 1.44 to 2.16; firth logistic regression $P = 3.55 \times 10^{-8}$ without eye color covariate, vs OR = 1.76, 95% CI = 1.43 to 2.17; $P = 9.25 \times 10^{-8}$, with eye color covariate; Figure 3, C; Supplementary Table 4, available online). Conversely, the odds ratio of *HERC2* risk SNP rs12913832 lost statistical significance with eye color covariate (OR = 0.57, 95% CI = 0.48 to 0.67; $P = 1.88 \times 10^{-11}$, without eye color covariate, vs OR = 0.76, 95% CI = 0.57 to 1.02; $P = 0.06$, with eye color covariate), in accordance with the major role of rs12913832 in the determination of eye pigmentation (17,18). As expected, the odds ratio of *CLPTM1L*, a gene with no known role in pigmentation, remained unchanged (rs421284: OR = 1.58, 95% CI = 1.35 to 1.86; $P = 1.98 \times 10^{-8}$, without eye color covariate vs OR = 1.58, 95% CI = 1.34 to 1.86; $P = 4.01 \times 10^{-8}$, with eye color covariate; Figure 3, C; Supplementary Table 4, available online). This indicates that the implication of the *IRF4* locus in UM risk not only is explained by the prevalence of UM among individuals with light eye color but also points toward another role for this risk locus beyond pigmentation.

## Pigmentation Risk Loci and UM Epidemiology

The higher prevalence of UM among individuals of European ancestry strongly supports the existence of inherited risk alleles for the disease. The *TERT/CLPTM1L* risk locus does not account for this population bias, as the risk haplotype is more frequent in African American populations than those of European ancestry (rs421284: VAF = 0.597 vs 0.429, respectively) (Supplementary Table 5, available online; Genome Aggregation Database v2.1). However, the risk haplotypes of both *IRF4* and *HERC2* are found at statistically significantly higher frequencies in populations of non-Finnish European ancestry (NFE) than in those of African or African American and East Asian origins (populations defined by Genome Aggregation Database) (*IRF4* rs12203592: VAF = 0.144, 0.034, and 0.000, respectively; *HERC2* rs12913832: VAF = 0.803, 0.125, and 0.001, respectively; 2-sided Fisher test $P < 1.00 \times 10^{-20}$ for all statistical comparisons of NFE vs East Asian and NFE vs African and African or African American). Therefore, the higher frequency of the risk alleles of these 2 pigmentation loci may at least partly explain the higher prevalence of UM in European populations.

## Association Study for the Two Major UM Subtypes

Loss of chromosome 3 is the strongest factor associated with poor metastatic outcome in UM and correlates with increased mortality (2,3). The genomic status was available for 384 UM patients, allowing us to test for differential association of UM risk loci according to chromosome 3 status. Association studies

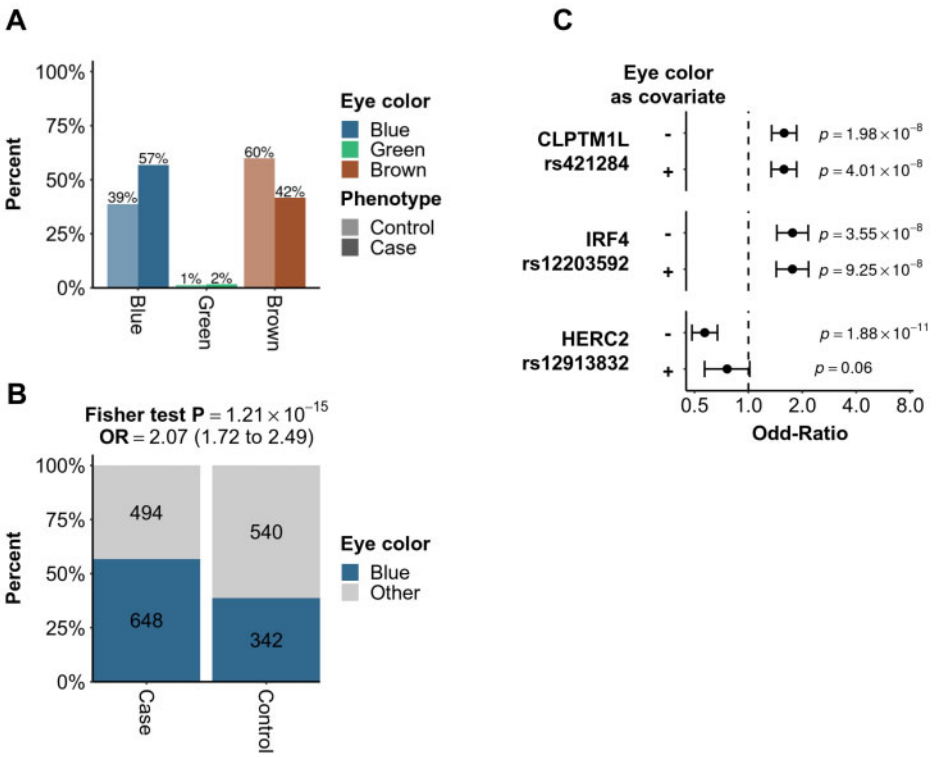


**Figure 3.** Eye pigmentation and uveal melanoma risk. **A)** Proportion of blue, green, and brow eye colors among uveal melanoma (UM) patients (**dark shade**) and controls (**light shade**), as predicted by the IrisPlex System (18). **B)** Proportion of blue eyes vs other eye colors in UM patients and controls. The number of individuals is indicated. The association of blue eye color with UM risk is indicated by the Fisher test *P* value and odds ratio (OR). The 95% confidence interval for the odds ratio is indicated within brackets. **C)** Effect of eye color as a GWAS covariate on the odds ratio for the 3 main SNPs of statistically significant UM risk loci (*CLPTM1L*, *IRF4*, and *HERC2*). The error bars indicate the 95% confidence intervals for the odds ratio. Statistical significance was assessed using a 2-sided Fisher test. The + and - indicate the inclusion or exclusion of eye color as a GWAS covariate, respectively. For each SNP and in both covariate conditions, association with UM risk is represented by the odds ratio (**x-axis**) and associated *P* value. The **vertical dotted line** is set at odds ratio = 1.00, indicating an absence of association with UM. All statistical tests were 2-sided. *CLPTM1L* = cleft lip and palate transmembrane protein 1-like; *HERC2* = HECT and RLD domain containing E3 ubiquitin protein ligase 2; *IRF4* = interferon regulatory factor 4.

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were performed independently on UMs with D3 or M3 (246 M3 and 138 D3) vs controls (CTL), for the most statistically significant SNP of each risk locus identified by GWAS (Table 1). Interestingly, rs12203592 (*IRF4* locus) showed a strong association with D3 UM, using a logistic regression model ($OR_{D3vsCTL}$ = 2.73, 95% CI = 1.87 to 3.97; $P = 1.78 \times 10^{-7}$), whereas the association vanished completely in M3 UM ($OR_{M3vsCTL}$ = 1.01, 95% CI = 0.7 to 1.47; $P = .95$). On the contrary, rs12913832 (*HERC2* locus) showed a statistically significant high association with M3 UM but not with D3 UM ($OR_{M3vsCTL}$ = 2.43, 95% CI = 1.79 to 3.29; $P = 1.13 \times 10^{-8}$; $OR_{D3vsCTL}$ = 1.10, 95% CI = 0.80 to 1.52; $P = .56$). As for rs421284 (*CLPTM1L* locus), no preferential association was found in either UM subgroup ($OR_{D3vsCTL}$ = 2.26, 95% CI = 1.61 to 3.17; $P = 2.64 \times 10^{-6}$; $OR_{M3vsCTL}$ = 1.55, 95% CI = 1.18 to 2.03; $P = .001$) (Table 1). To further assess the statistical significance of the observed differential association of rs12203592 in M3 and D3, we compared both subgroups ($OR_{M3vsD3}$) for their association with UM risk SNPs (Supplementary Table 6, available online). As expected, the odds ratio of *CLPTM1L* rs421284 with M3 UMs or D3 UMs collapsed toward the value 1, indicating that this SNP was similarly associated with both subgroups ($OR_{M3vsD3}$ = 0.86, 95% CI = 0.67 to 1.11; $P = .33$). Conversely, the low odds ratio M3 vs D3 and statistically significant *P* value obtained for *IRF4* rs12203592 ($OR_{M3vsD3}$ = 0.38, 95% CI = 0.27 to 0.52; $P = 8.46 \times 10^{-7}$) and the high odds ratio M3 vs D3 for *HERC2* rs12913832 ($OR_{M3vsD3}$ = 1.81, 95% CI = 1.38 to 2.38; $P = 3.87 \times 10^{-4}$) recapitulated the specific association of these risk regions for D3 UM and M3 UM, respectively.

These data strongly suggest that UM tumor biology is influenced by the genetic background predisposing to UM, with *CLPTM1L* SNPs predisposing to all UM types, *IRF4* SNP predisposing specifically to risk in D3 UM, and *HERC2* locus to risk in M3 UM.

## Discussion

We extended our initial UM GWAS by including 1142 UM patients and performing genome-wide genotype imputation. This allowed us to recapitulate the previously described *CLPTM1L* risk locus and to further identify *IRF4* and *HERC2*, 2 pigmentation loci, as UM genetic risk factors. Furthermore, we demonstrated that whereas *CLPTM1L* is a risk locus in all UM subgroups, *IRF4* is specifically associated with D3 UM and *HERC2* specifically with M3 UM.

The *TERT/CLPTM1L* region has frequently been associated in GWAS studies, with higher and lower tumor risk depending on cancer types (20). The function of CLPTM1L is not yet fully understood, but this protein is thought to contribute to RAS-dependent transformation and tumorigenesis, including in pancreatic tumorigenesis (21-23). On the other hand, TERT (on the same locus) plays a major role in telomere maintenance (24). In a previous study, we revealed a correlation between rs421284

**Table 1.** Main risk loci in uveal melanoma according to their chromosome 3 status

| ID[a] | SNP[b] | Symbol | Alternative allele | Monosomy 3 | | | Disomy 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Total No. (patients/controls) | OR (95% CI) | P[c] | Total No. (patients/controls) | OR (95% CI) | P[c] |
| 5:1325590: T > C | rs421284 | *CLPTM1L* | C | 1126 (244/882) | 1.55 (1.18 to 2.03) | 0.001 | 1018 (137/881) | 2.26 (1.61 to 3.17) | $2.64 \times 10^{-6}$ |
| 6:396321: C > T | rs12203592 | *IRF4* | T | 1126 (244/882) | 1.01 (0.70 to 1.47) | 0.95 | 1018 (137/881) | 2.73 (1.87 to 3.97) | $1.78 \times 10^{-7}$ |
| 15:28365618: A > G | rs12913832 | *HERC2* | G | 1126 (244/882) | 2.43 (1.79 to 3.29) | $1.13 \times 10^{-8}$ | 1018 (137/881) | 1.10 (0.80 to 1.52) | 0.56 |

[a]ID refers to chromosome number: chromosomal genomic position: reference allele > alternative allele, based on genome build GRCh37 (hg19). CI = confidence interval; OR = odds ratio.
[b]SNP = single nucleotide polymorphism, according to the Single Nucleotide Polymorphism Database.
[c]Two-sided P values were calculated by general linear model.

genotype and *CLPTM1L* expression but not *TERT*, the latter being poorly expressed in UMs (12). Whether *CLPTM1L* or *TERT* is the target of this risk haplotype in UM tumorigenesis is still unclear.

We confirmed the association of the *OCA2/HERC2* locus with UM risk, initially identified as candidate SNPs by Ferguson et al. (25). We confirmed the correlation between *HERC2* rs12913832 and *OCA2* expression in UM, with a decreased expression in individuals carrying the G allele (Supplementary Figure 6, C, available online). HERC2 is known to regulate the expression of *OCA2*, which codes for a protein involved in determining the melanin type and amount (26). These 2 genes are the main genetic determinants of iris color (18). In melanocytic cell lines, the transcription factor HLTF binds to the A but not the G allele of rs12913832, creating an activating loop for *OCA2* transcription by the recruitment of MITF and LEF1 (17,27). The rs12913832 A allele is consequently associated with high expression of *OCA2*, production of melanin, brown eye color, and low UM risk, and conversely for the rs12913832 G allele.

The third UM risk locus identified in the present study is characterized by a single risk SNP on *IRF4*, rs12203592 (25). IRF4 regulates the expression of key pigmentation genes in association with MITF, including *TYR* involved in the production of melanin. The *IRF4* locus is also associated with melanocytic naevus count, freckling, and tanning ability (28-30). TFAP2$\alpha$ recognizes rs12203592 C allele in melanocytes, allowing the recruitment of MITF, YY1, and potentially LEF1 and increasing *IRF4* expression (15,16). Conversely, rs12203592 T allele prevents TFAP2$\alpha$ binding resulting in lower *IRF4* expression. We showed that the rs12203592 UM risk allele T is associated with a dramatic decreased expression of *IRF4* (Supplementary Figure 5, A, available online). Of note, only a minority of individuals (3 in our in-house series) carry the TT genotype. A similar eQTL pattern was reported in sun-exposed skin from Genotype-Tissue Expression, whereas an opposite direction was found in other tissues (Supplementary Figure 5, B, available online), strongly suggesting that *IRF4* is regulated in a tissue-specific manner.

The present GWAS demonstrates the role of 2 pigmentation genes in the genetic risk of UM, in addition to the *CLPTM1L/TERT* risk locus. This is consistent with light iris color being a risk factor for UM (OR = 1.75) (11,19,31) similar to our finding (OR = 2.07). Iris pigmentation depends on the production and maturation of melanin as well as on the ratio of the 2 types of melanin: eumelanin (black-brown, densely packed) and pheomelanin (yellow-to-red, loosely packed). Melanin plays a major role in protecting against ultraviolet radiation (UVR) by absorbing free radicals and inhibiting UV-mediated damage (32). Pheomelanin, however, can also induce more oxidative damage on UVR than eumelanin (33), which was proposed to explain the contribution of light iris color in UM (34). However, the steady UM incidence despite increased UVR exposure, the low tumor mutation burden, and absence of UVR mutational signature in UM tumors ruled out this hypothesis (5,35). Interestingly, iris melanoma, a rare form of UM, is associated with high tumor mutation burden and a UVR signature (36), consistent with iris color being a risk factor for iris melanoma (37). However, our GWAS is restricted to choroid melanoma, a tissue that, unlike the iris, is not directly exposed to sunlight. In this respect, *IRF4* and potentially *HERC2/OCA2* SNPs may play a role outside from iris pigmentation to explain UM risk. However, a limitation of our study is that eye pigmentation is deduced from genotypes, which are also risk SNPs for UM, making it challenging to derive causal statements.

Status of chromosome 3 and *BAP1* delineates 2 UM subtypes, M3 and *BAP1*-inactivated high-risk tumors and D3 and wild-

type *BAP1* low-risk tumors (2-4,8). Strikingly, whereas *CLPTM1L* region confers similar susceptibility for M3 UM and D3 UM, we show that the risk for M3 UM is associated with the *OCA2/HERC2* region and D3 UM with the *IRF4* locus. How these processes influence the malignant transformation is unknown but most probably independent of the protective role of melanin against UVR. Furthermore, our data reinforce the idea that UM encompasses at least 2 diseases, with distinct clinicobiological characteristics (8,38-40) and distinct susceptibility loci.

Further studies should investigate the molecular mechanisms behind these UM genetic susceptibility loci to understand the role of pigmentation genes in UM risk. This study provides important insights in the genetics of UM and may lead to improvements in risk prediction and to a better understanding of the biological basis of UM.

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## Notes

**Role of the funders:** The funders had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

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**Author contributions:** LM, A-CD, and AH contributed equally to this study. LM, A-CD, AH, and M-HS conceptualized the study and developed its methodology. G C-T, OC, and GS provided resources (GWAS control samples). A-CD, LM, AB, and J-FD, conducted research investigation (experiments). LM, AH, TV, and JN performed data curation and formal analysis. GP, NC, and MM provided resources. A-CD, AH, and LM conducted experiments, performed visualization/data presentation, and wrote and edited the manuscript. GC, MR, JN, and MR reviewed the manuscript. M-HS supervised the study. All authors reviewed and approved the final manuscript.

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## Data Availability

Dataset2 and 3 genotyping data used in the analysis have been deposited and are available on the European Genome-Phenome Archive (EGA) (https://ega-archive.org/) under accession number EGAS00001005200. Previously published genotyping of dataset1 patients and controls are found on EGA under Accession number EGAS00001002334 and on the database for Genotypes and Phenotypes (dbGaP) under accession number phs001271.v1.p1., respectively. Previously published expression data (RNA-seq data) of 73 UM tumors are available at EGA under accession no. EGAS00001002932). PCAs were performed using HapMap3 (ftp://ftp.ncbi.nlm.nih.gov/hapmap/genotypes/hapmap3_r3). For expression quantitative trait loci (eQTL) analyses, data was obtained from the Genotype-Tissue Expression (GTEx) public database (https://www.gtexportal.org/home/). Allele frequency of SNPs of interest in different populations was obtained from the Genome Aggregation Database (GnomAD v2.1.1, https://gnomad.broadinstitute.org).

Code availability: The following web-based resources were used in the GWAS analysis: PLINK 1.9 and 2.0 (https://www.cog-genomics.org/plink/1.9/, https://www.cog-genomics.org/plink/2.0/), Michigan Imputation Server (https://imputationserver.sph.umich.edu/index.html), GitHub (https://github.com/DReichLab/EIG), and HIrisPlex (https://hirisplex.erasmusmc.nl/). The code underlying this article will be shared on reasonable request to the corresponding author.

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