Genetic 3′UTR variation is associated with human pigmentation characteristics and sensitivity to sunlight

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Abstract
Sunlight exposure induces signalling pathways leading to the activation of melanin synthesis and tanning response. MicroRNAs (miRNAs) can regulate the expression of genes involved in pigmentation pathways by binding to the complementary sequence in their 3′untranslated regions (3′UTRs). Therefore, 3′UTR SNPs are predicted to modify the ability of miRNAs to target genes, resulting in differential gene expression. In this study, we investigated the role in pigmentation and sun-sensitivity traits, as well as in melanoma susceptibility, of 38 different 3′UTR SNPs from 38 pigmentation-related genes. A total of 869 individuals of Spanish origin (526 melanoma cases and 343 controls) were analysed. The association of genotypic data with pigmentation traits was analysed via logistic regression. Web-based tools for predicting the effect of genetic variants in microRNA-binding sites in 3′UTR gene regions were also used. Seven 3′UTR SNPs showed a potential implication in melanoma risk phenotypes. This association is especially noticeable for two of them, rs2325813 in the MLPH gene and rs752107 in the WNT3A gene. These two SNPs were predicted to disrupt a miRNA-binding site and to impact on miRNA-mRNA interaction. To our knowledge, this is the first time that these two 3′UTR SNPs have been associated with sun-sensitivity traits. We state the potential implication of these SNPs in human pigmentation and sensitivity to sunlight, possibly as a result of changes in the level of gene expression through the disruption of putative miRNA-binding sites.

KEYWORDS
3′untranslated region, microRNA, naevus, SNP, solar lentigines

1 | INTRODUCTION

Cutaneous melanoma incidence is increasing rapidly among white-skinned populations. Factors that are mainly involved in the aetiology of melanoma are not only of pigmentary/genetic nature, but also of environmental nature. Chronic sun exposure thus plays a key role in causing melanoma through DNA damage. Ultraviolet (UV) exposure stimulates the synthesis of melanin in melanosomes via activation of human pigmentation pathways, with the aim of protecting skin from the harmful effects of sunlight. Gene expression can be regulated by a wide range of mechanisms. Recently, post-transcriptional regulatory processes—specifically controlled by mRNA-binding factors—have emerged as a fundamental...
and effective cellular mechanism to regulate gene expression, and alterations in these processes can cause numerous pathologies including immunological disease,[15] neurodegeneration[16] and tumor development.[17,18] Therefore, differential gene expression may be as important for disease susceptibility as non-synonymous genetic changes.

Among the mRNA-binding factors, microRNAs (miRNAs)—short non-coding RNA molecules (22-24 nt) encoded by intronic or intergenic sequences—act as key gene regulators by repressing mRNA translation or by destabilizing/degrading mRNAs in the cytoplasm, via perfect or imperfect binding to their complementary base pair sequence in the 3′untranslated region (3′UTR) of the mRNA target.[19] Therefore, the 3′UTR region is emerging as critically important in regulating gene expression,[17] and polymorphisms in the miRNA-binding sites of the 3′UTR of genes may alter the binding efficiency and miRNA-mRNA gene expression regulation. In support of this hypothesis, recent studies have identified variants in the 3′UTR of genes that increase the susceptibility to melanoma,[20] lung,[21] colorectal[22] and ovarian cancer[23] by affecting the ability of miRNAs to bind. In particular, two sequence changes in the 3′UTR of the CDKN2A gene have been significantly correlated with melanoma risk,[24] but also with a shorter progression time from primary to metastatic melanoma.[25]

Here, we hypothesize that differences identified in nucleotide composition of 3′UTRs SNP sites of genes previously associated with pigmentation and/or skin cancer can be a reason for causing differences in human pigmentation, sensitivity to sunlight and thus in melanoma susceptibility. In this study, we describe the role of 38 different 3′UTR polymorphisms from 38 different candidate pigmentation and melanoma susceptibility genes in a population of Spanish origin. Additionally, we use miRNA-binding prediction tools to identify variants affecting putative miRNA-binding sites and to predict their impact on miRNA-mRNA interaction.

2 | METHODS

2.1 | Study subjects and data collection

A total of 526 melanoma cases and 343 cancer-free controls were included in this study. Melanoma cases were recruited at the Departments of Dermatology of four Spanish hospitals: Gregorio Marañon General University Hospital (Madrid), La Paz University Hospital (Madrid), Ramon y Cajal University Hospital (Madrid) and Castellon Province Hospital (Castellon). Volunteer cancer-free control samples were recruited from the Madrid College of Lawyers, Gregorio Marañon Hospital, Valencia Clinic Hospital and Castellon Province Hospital. We carefully selected all cases and controls included in this study to account for confounding variables. As far as it was possible, controls were frequency-matched to the cases by age, sex and place of birth. All individuals were Caucasians of Spanish origin with the same genetic background, because there is evidence of high genetic homogeneity within different Spanish geographical regions.[26]

Each participant completed a standardized questionnaire to collect information on sex, age, pigmentation characteristics (eye colour, hair colour, skin colour, number of naevi and presence of solar lentigines), history of childhood sunburns, and personal and family cancer history.

Genomic DNA from cases and controls was isolated from peripheral blood lymphocytes using the traditional saline method, the DNAzol procedure (Invitrogen, Eugene, OR, USA) or the MagNA Pure LC Instrument according to the manufacturer’s protocol (Roche Molecular Biochemicals AQ2, Mannheim, Germany). DNA concentration was quantified in samples before genotyping using a Nanodrop 2000 spectrophotometer or Quant-IT PicoGreen dsDNA Reagent (Invitrogen). Genomic DNA was amplified using the Genomiphi DNA Amplification Kit (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Samples were diluted to a final solution of 50 ng/mL and stored at −20°C.

The study was approved by the Ethics Committee of the Biomedical Research Institute—INCLIVA (Valencia, Spain). Written informed consent was obtained from all participants.

2.2 | SNP selection

Previous literature and information from public databases were used to perform our candidate gene list. We selected genes previously associated with pigmentation pathways and/or melanoma risk,[7,9,27,28] preferably including direct targets of functional miRNA that happen to be deregulated in melanoma. Ensembl BioMart (http://www.ensembl.org/biomart/martview) was used to retrieve germ line variants from all genes selected. Filters were used to ensure that all SNPs were located within the 3′UTRs. SNP codes, locations, minor and ancestral alleles and their frequencies were obtained from the NCBI (www.ncbi.nlm.nih.gov/SNP), HapMap (www.hapmap.org) and Ensembl Variation (www.ensembl.org/info/genome/variation) databases. From the data retrieved, Haploview v4.2 was used to identify tag-SNPs that optimally capture allelic variation among SNPs, using a pairwise SNP approach with a minimum r2 threshold of 0.8.[29] To ensure a high genotyping success rate, a minor allele frequency (MAF) threshold of 0.1 in the Caucasian population from the International 1000 Genomes Project (http://www.1000genomes.org/) was established in the SNP selection process. Forty-five tag-SNPs were finally selected.

2.3 | Genotyping

SNP genotyping was conducted by the Spanish National Genotyping Centre (CeGen-PRB2, Santiago de Compostela) as a contract service using the iPLEX Gold MassARRAY technology, according to manufacturer’s protocol (Sequenom, San Diego, CA, USA). All assays were performed in 384-well plates, including a negative control and a trio of Coriell samples (Na10860, Na10861 and Na11984) for quality control. Genotyping specificity was assessed by adding three DNA duplicates (two intra-assays and one interassay) per plate, yielding 100% consistent replication results. In addition, cases and control samples were always included in the same run. SNPs with a genotyping rate lower than 90% (10% missing data) were excluded for further analysis.
Identification of potential microRNA-binding sites

The potential effect of 3′UTR polymorphisms on miRNA binding was examined using MirSNP (http://cmbi.bjmu.edu.cn/mirsnp) and miRNASNP (http://www.bioguo.org/miRNASNP/).

MiRNASNP employs the miRanda target prediction algorithm (http://www.microrna.org) with stringent 7-nt seed site pairing as major criteria for prediction consistency. To increase precision, we only considered target sites with an alignment score cut-off ≥140, energy cut-off ≤−10 kcal/mol and miRSVR score ≤−0.1.

MiRNASNP uses two miRNA target prediction tools: TargetScanHuman (http://www.targetscan.org/) and miRanda. MiRNASNP also incorporates RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid) to quantify the binding energy changes in the interaction of miRNAs with the wild-type target sequence compared to the derived 3′UTR sequence. Only the duplexes with hybridization free energy s−20 kcal/mol were chosen.

Identification of validated pathways targeted by in silico predicted microRNAs

In order to further investigate the miRNAs predicted to bind to the two 3′UTR SNPs highly associated with phenotypic traits (hsa-miR-149-5p, hsa-miR-892b, hsa-miR-185-3p and hsa-miR-762), we used DIANA-miRPath v2.0 (http://www.microrna.gr/miRPathv2) to identify the miRNA-targeted pathways. The output provides intuitive heat maps and enriched KEGG pathway visualizations for easier inspection.

In silico quantitative analysis of tissue-specific expression

Data from the Genotype-Tissue Expression (GTEx) project (dbGaP accession no. phs000424.v6.p1) were used for external validation and to evaluate differential tissue-specific gene expression regarding 3′UTR SNP genotypes (http://www.gtexportal.org/home/).

Statistical analysis

For each polymorphism studied, Fisher’s exact test was used both to check for deviations from Hardy-Weinberg equilibrium (HWE) among controls and to compare differences in allele counts between cases and controls. To account for differences between populations, allele frequencies of our Spanish population were compared to those of both a North European population (CEU) and a Southern one from Tuscany (TSI) using Fisher’s exact test.

Associations between the genotyped genes and various pigmentation variables. This was performed for four different patterns of inheritance: dominant (major homozygotes vs heterozygotes plus minor homozygotes), overdominant (major homozygotes plus minor homozygotes vs heterozygotes), recessive (major homozygotes plus heterozygotes vs minor homozygotes) and additive (counting additively for each copy of minor allele). Genotype-related odds ratios (ORs), their corresponding 95% confidence intervals (CIs) and associated P-values were estimated. Association analyses with phenotypic traits were adjusted by sex, because sex-differentiated allelic effects for pigmentation traits, sensitivity to sunlight and melanoma have been previously shown.

To assess associations among genotypes and melanoma risk, genotype-related ORs, their corresponding 95% CIs and associated P-values were estimated via unconditional logistic regression. Multivariate logistic regression was also carried out combining sex and all significant risk factors revealed in Table S1. This was also performed for all four patterns of inheritance.

Statistical analyses and plots were conducted using R statistical framework (http://www.R-project.org). All genetic analyses were performed estimating the effect of the minor allele in the Spanish population. Unknown and missing values were excluded at each specific analysis. All P-values were two-sided, and those less than 0.05 were considered statistically significant.

RESULTS

The role of 38 polymorphisms in as many pigmentation and melanoma susceptibility genes was initially investigated. No evidence of departure from HWE for any of the 38 SNPs was found. Two 3′UTR polymorphisms revealed differences in minor allele frequencies (MAFs) between cases and controls: ADAMTS20 rs6582463 and HOXB7 rs15689. We did not observe differences in MAFs between cases and controls for any other SNP (Table S2).

We compared Spanish allele frequencies to those of CEU and TSI subjects, using the 1000 Genomes Project (phase 3) allele counts as the reference (Table S2). Spanish MAFs differed significantly from CEU frequencies in three SNPs (7.89%): rs4733967 (ADAM9), rs3212369 (MC1R) and rs1690916 (MDM2). Seven SNPs presented different allele frequencies from those reported in TSI population data: rs6582463 (ADAMTS20), rs742106 (DTNB1), rs12952 (EXOC2), rs8022 (KIT), rs995030 (KITLG), rs14983 (MMP7) and rs1551306 (TPCN2). In spite of these differences, allele frequencies in Spain were very similar to those from both a North European population (CEU) and a Southern one (TSI), with a high correlation (R2) of .916 and .913, respectively (Figure S1).

Association analysis

Evidence of association with phenotypic characteristics for the thirty-eight 3′UTR SNPs was assessed. Considering a P-value threshold of .05, 17 SNPs were associated with at least one sun response trait, and 11 SNPs showed association with at least one pigmentation trait (Figure 1). Among them, we further investigated the 7 SNPs that
presented the most potential allelic effects for phenotypic traits in the Spanish population (P-value<0.01). The rs2325813 SNP, located in the MLPH gene, was correlated with the presence of more than 50 naevi (P=8.97×10^-5). Two SNPs, HOXC8 rs4142680 and WNT3A rs752107, correlated with the presence of lentigines (P=6.57×10^-3 and P=4.53×10^-3, respectively), while LYST rs6696123 showed association with an absence of lentigines (P=2.56×10^-5). Two more SNPs, rs10270 in the CLIP1 gene and rs4980113 in the KCNMA1 gene, were associated with dark hair colour (P=1.44×10^-5 and P=2.67×10^-5, respectively). Finally, KIT rs8022 was correlated with light eye colour (P=8.88×10^-5) (Table 1).

Likewise, we carried out an association analysis between genotypes and melanoma risk. Five SNPs showed a tendency to correlate with melanoma susceptibility in the Spanish population. Among them, three SNPs (HOXB7 rs1589, MARCKS rs28558559 and ADAM9 rs473967) showed a melanoma protective effect (OR<1). On the other hand, PTC2H2 rs41269085 and ADAMTS20 rs6582463 displayed a melanoma risk effect (OR>1) (Table S3).

For the association results to be adjusted by the confounding variables, we performed a multivariate analysis including phenotypic risk factors (hair colour, solar lentigines and the presence of childhood sunburn) and sex as covariates. Polymorphisms located in HOXB7, MARCKS, ADAM9 and PTCH2 remained significant after the adjustment, with no substantial changes in allelic effects, confirming the putative role of these variants in melanoma susceptibility. Additionally, KCNMA1 rs4980113 and IRF4 rs9391997 were marginally associated with melanoma protection (Table S3).

### 3.2 Variants affecting microRNA-binding sites in human pigmentation

All 3'UTR polymorphisms that presented association with phenotypic characteristics and/or melanoma were analysed by two specialized web-based programmes for predicting miRNA-binding sites in the 3'UTR.

Cross-prediction was required for verifying the predicted target sites. After applying all sequential filtering steps, eight of all 3'UTR polymorphisms evaluated had at least one miRNA predicted to bind (Table 2). Three 3'UTR variants interrupted miRNA-mRNA interaction or reduced miRNA-mRNA interaction by increasing the free energy of the corresponding duplexes after the minor allele introduction in the target sequence. Conversely, three variants created new miRNA target sequences or enhanced miRNA-binding efficiency by decreasing hybridization free energy. Two variants both disrupted/decreased and created/enhanced multiple miRNA target sequences in the sequences studied (Table 2).

Once miRNAs of interest were identified using binding prediction tools, we used an in silico approach to identify pathways that are under the regulation of the predicted miRNA signature. The four selected miRNAs and the targeted KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways are displayed in Figure 2. Among all the significant targeted KEGG pathways, we identified three of them involved in pigmentation and skin cancer: "Wnt signalling pathway-hsa04310" (P=4.24×10^-3), "MAPK signalling pathway-hsa04010" (P=1.07×10^-4) and "basal cell carcinoma-hsa05217" (P=2.52×10^-3). Figure S2 represents in detail these KEGG pathways, highlighting the specific target genes of the selected miRNAs.

We further evaluated the association between the genotype of both MLPH rs2325813 and WNT3A rs752107 and the gene expression levels in sun-exposed skin using the GTEx portal. Individuals carrying rs752107*T allele, which was predicted to decrease miRNA-mRNA-binding efficiency, seem to present increased expression of WNT3A in sun-exposed tissue (Figure S3, Figure S4). No changes in MLPH expression regarding genotype were observed.
### Table 1

3′UTR variants highly associated with phenotypic traits in the Spanish population (*P*-value < .01)

| Trait    | Gene   | SNP rs#   | Genotype | Protective phenotype N (%) | Risk phenotype N (%) | Inheritance mode | OR (95% CI)       | *P*-value |
|----------|--------|-----------|----------|-----------------------------|----------------------|------------------|-------------------|-----------|
| Naevi    | MLPH   | rs2325813 | TT       | 591 (82.3)                  | 75 (69.4)            | Additive         | 2.03 (1.36-3.02)  | 8.97E-04 |
|          |        |           | CT       | 121 (16.9)                  | 29 (26.9)            |                  |                   |           |
|          |        |           | CC       | 6 (0.8)                     | 4 (3.7)              |                  |                   |           |
| Lentigines | WNT3A  | rs752107  | CC       | 196 (56.2)                  | 216 (45.3)           | Overdominant     | 1.66 (1.25-2.21)  | 4.53E-04 |
|          |        |           | CT       | 118 (33.8)                  | 218 (45.7)           |                  |                   |           |
|          |        |           | TT       | 35 (10.0)                   | 43 (9.0)             |                  |                   |           |
| Lentigines | LYST   | rs6696123 | TT       | 100 (28.6)                  | 182 (38.1)           | Additive         | 0.73 (0.60-0.90)  | 2.56E-03 |
|          |        |           | CT       | 184 (52.6)                  | 231 (48.3)           |                  |                   |           |
|          |        |           | CC       | 66 (18.9)                   | 65 (13.6)            |                  |                   |           |
| Lentigines | HOXC8  | rs4142680 | TT       | 138 (39.4)                  | 160 (33.6)           | Overdominant     | 1.47 (1.11-1.94)  | 6.57E-03 |
|          |        |           | CT       | 143 (40.9)                  | 240 (50.4)           |                  |                   |           |
|          |        |           | CC       | 69 (19.7)                   | 76 (16.0)            |                  |                   |           |
| Hair colour | CLIP1  | rs10270   | GG       | 328 (46.1)                  | 83 (56.8)            | Overdominant     | 0.55 (0.37-0.80)  | 1.44E-03 |
|          |        |           | AG       | 321 (45.1)                  | 45 (30.8)            |                  |                   |           |
|          |        |           | AA       | 63 (8.8)                    | 18 (12.3)            |                  |                   |           |
| Hair colour | KCNMA1 | rs4980113 | GG       | 182 (25.5)                  | 47 (32.2)            | Overdominant     | 0.57 (0.40-0.83)  | 2.67E-03 |
|          |        |           | CG       | 377 (52.9)                  | 57 (39.0)            |                  |                   |           |
|          |        |           | CC       | 154 (21.6)                  | 42 (28.8)            |                  |                   |           |
| Eye colour | KIT    | rs8022    | GG       | 416 (73.5)                  | 229 (80.9)           | Overdominant     | 0.62 (0.43-0.89)  | 8.88E-03 |
|          |        |           | GT       | 139 (24.6)                  | 48 (17.0)            |                  |                   |           |
|          |        |           | TT       | 11 (1.9)                    | 6 (2.1)              |                  |                   |           |

SNP, single nucleotide polymorphism; N, number of individuals; %, percentage of individuals per group among the total; OR, odds ratio per minor allele; CI, confidence interval.

### Table 2

Candidate microRNAs predicted to bind to 3′UTR SNPs showing association with pigmentation traits, sensitivity to sunlight and melanoma susceptibility

| Gene     | 3′UTR SNP rs# | Allele change | mRNA predicted to bind to the target site | Effect on miRNA binding | Free energy of miRNA-mRNA binding for WT (kcal/mol) | Free energy of miRNA-mRNA binding for MA (kcal/mol) | Energy change (kcal/mol) |
|----------|---------------|---------------|-------------------------------------------|-------------------------|-----------------------------------------------------|---------------------------------------------------|--------------------------|
| DTNBP1   | rs742106      | G→A           | hsa-miR-1293                              | Decrease                | −26.40                                              | −23.80                                            | −2.60                    |
| E2F1     | rs3213180     | C→G           | hsa-miR-1182                              | Break                   | −31.30                                              | 0.00                                              | −31.30                   |
| FOXO3    | rs9400241     | A→C           | hsa-miR-2115-5p                           | Break                   | −28.40                                              | 0.00                                              | −28.40                   |
| KIT      | rs8022        | G→T           | hsa-miR-548as-3p                          | Create                  | 0.00                                                | −20.80                                            | 20.80                    |
| MLPH     | rs2325813     | T→C           | hsa-miR-185-3p                            | Enhance                 | −29.00                                              | −31.70                                            | 2.70                     |
| SOX9     | rs1042667     | A→C           | hsa-miR-1181                              | Enhance                 | −28.80                                              | −31.50                                            | 2.70                     |
| WNT3A    | rs752107      | C→T           | hsa-miR-149-5p                            | Decrease                | −29.90                                              | −27.60                                            | −2.30                    |

SNP, single nucleotide polymorphism; 3′UTR, 3′ untranslated region; WT, wild-type target allele; MA, minor allele target allele.

*The prediction of miRNA-binding sites was performed using MirSNP and miRNASNP

*The effect of the SNP on miRNA binding was given by MirSNP. These effects can be classified following four categories: (a) decrease—reduction in the binding efficacy, (b) enhance—increase in the binding efficacy, (c) break—disruption of the binding site or (d) create—creation of a new binding site.

*The free energy value of miRNA-mRNA binding was obtained from miRNASNP.

*Energy change (kcal/mol) indicates difference in minimum free energy of binding before and after introduction of the minor allele.
variants with putative phenotypic implications. As 3′UTRs are critical regulatory elements in gene expression,[43,44] polymorphisms located in this region of genes associated with pigmentation pathways may contribute to pigmentation characteristics and sensitivity to sunlight, as well as to melanoma susceptibility.

This study allowed us to observe interesting associations between genotypic and phenotypic traits in our population. Despite detecting several candidate 3′UTR SNPs with a potential implication in pigmentation and sensitivity to sunlight, we could not validate them because associations did not reach genomewide nor candidate gene levels of statistical significance. Perhaps our restricted sample size resulted in limited statistical power to detect unequivocal associations for these SNPs. Replication of our findings in a larger study is therefore essential before drawing any firm conclusion. It is noted that adjusting analyses by sex has conferred strength to our results, excluding bias from the sexual disparity in pigmentation and melanoma incidence and outcome observed in previous studies.[38-40,42,43]

The first interesting finding was the reasonably strong association of rs2325813, located in the 3′ UTR of the MLPH gene, with high naevus count. The human MLPH gene (OMIM #606526) has been shown to be involved in mature melanosome transport within melanocyte before being transferred to keratinocytes. MLPH encodes a member of the exophilin subfamily of Rab effector proteins known as melanophilin, which acts as a link between the small GTPase melanosome-bound RAB27A and the actin-associated motor protein MYO5A.[44] This protein complex plays a crucial role in the melanosome motility in melanocytes, and aberrations in any of the complex components have been shown to result in perinuclear localisation of melanosomes and therefore failure to transfer mature melanosomes to adjacent keratinocytes, eventually causing hypopigmentation.[45] Human individuals homozygous for a pathogenic MLPH mutation (c.102C>T; p.R35W) display Griscelli syndrome type 3, a pigmentary disorder characterized by a hypopigmented phenotype.[46-47] The naevus-associated SNP in this work, rs2325813, is predicted to disrupt a binding site of two miRNAs (hsa-miR-185-3p and hsa-miR-762). The presence of the minor allele in the target sequence enhances miRNA-binding efficiency, repressing miRNA translation of MLPH, and ultimately limiting the formation of RAB27A/melanophilin/myosin-5a complex. Thus, reduction in MLPH gene expression may cause an abnormal accumulation of mature melanosomes around the nucleus of melanocytes, resulting in light pigmentation and poor tolerance to sunlight. Interestingly, our results are consistent with the well-known correlation between melanocytic naevus number, a main risk-prediction factor for melanoma incidence, and the propensity to burn, rather than tan, of light-skinned individuals.[48]

Therefore, genes implicated in functions related with melanosome trafficking, especially the RAB27A/melanophilin/myosin-5a membrane transport pathway, would be relevant candidates for additional investigation in further pigmentation and melanoma studies.

WNT/β-catenin signalling has a pivotal role in the formation of melanocytes, because this pathway has been implicated in promoting the development of neural crest-derived melanocytes.[49,50] In humans, the WNT pathway is significantly upregulated in solar lentigines, suggesting that overstimulation of melanocyte proliferation and differentiation plays a crucial role in the pathogenic mechanism of solar lentigines.[51] Interestingly, in this work we identify a polymorphism, rs7352107, located in the 3′ UTR of the WNT3A gene that is strongly associated with the presence of solar lentigines. WNT3A (OMIM #606359) encodes a WNT ligand that acts through the WNT/β-catenin pathway promoting melanocyte differentiation, and may promote melanoma differentiation as well.[49,50] Furthermore, the minor allele of rs7352107 is predicted to decrease the binding efficiency to the 3′ UTR gene region of two microRNAs (hsa-miR-149-5p and hsa-miR-892b), leading to a weaker miRNA-mRNA interaction and therefore a higher level of secreted WNT3A ligand. This probably enhances the activation of the WNT/β-catenin signalling and subsequently the proliferation of melanocytes. These observations, together with the results from Yamada and cols.,[51] suggest that abnormal regulation of melanogenesis via gene expression changes is expected to be involved in several pigmentary disorders and in melanoma risk phenotypes. Thus, studies focusing on the regulation of WNT/β-catenin signalling could potentially clarify the causal mechanisms of pathogenic hyperpigmentation and hypopigmentation conditions.

The miRNAs predicted to bind to MLPH rs2325813 (hsa-miR-185-3p and hsa-miR-762) and to WNT3A rs7352107 (hsa-miR-149-5p and hsa-miR-892b) seem to target genes involved in pigmentation mechanisms and skin cancer. Remarkably, out of all significant pathways, “Wnt signalling pathway” and “MAPK signalling pathway” were the only ones targeted by three of the four miRNAs. Furthermore, “Basal cell carcinoma” pathway was also targeted by hsa-miR-185-3p and hsa-miR-762. These observations may corroborate the importance of these miRNAs in both human pigmentation and skin cancer pathways. Based on GTEx project data, genes encoding for these miRNAs, except for hsa-miR-892b, are expressed in sun-exposed skin (Figure S3), confirming the expression of these miRNAs in skin tissue,
and suggesting a possible role of these miRNAs in skin regulation and function.

Additionally, five polymorphisms displayed a notable statistical association with phenotypic characteristics. Among these SNPs, we would like to highlight that the variant rs4142680, located in the 3′UTR of HOXC8, displays an interesting predisposition tendency towards sun-damaged phenotypes. The HOXC8 gene has been shown to be massively upregulated in melanoma cancerous cells as a consequence of diminished miR-196a levels, leading to an aggressive melanoma phenotype via the overexpression of several tumorigenic target genes.[52] Curiously, the web-based miRNA-binding prediction analysis in this work showed an intermediate free energy (−16.60 kcal/mol) for binding hsa-miR-4509 to the 3′UTR sequence containing the rs4142680*T allele, and predicted that presence of the C allele may break the putative binding site. Thus, the association between rs4142680*C and the presence of solar lentigines may be the result of increased HOXC8 expression that could be possibly promoting melanocyte proliferation.

In summary, we analysed the potential implications of 3′UTR polymorphisms in pigmentation, sensitivity to sunlight and skin cancer. A plausible cause of the action of these 3′UTR SNPs in the appearance of different sun-related benign pigmented skin lesions might be the differential gene expression attained by disrupting putative miRNA-binding sites. Specifically, we detected two potential associations with well-recognized skin cancer risk traits that modify miRNA-mRNA interactions: rs2325814 in the 3′UTR of the MLPH gene and rs752107 in the 3′UTR of the WNT3A gene. Future functional studies will be needed to determine the exact implications of these polymorphisms. In addition, we detected five genes that might contribute to pigmentation variation in our population. The fact that MLPH, LYST and CLIP1 functions have been related to intra-cellular membrane trafficking and pigment disorders reinforces the need to explore more deeply the role of melanosome transport pathways in pigmentation and tanning ability. Similarly, the study of genes that are at least partially involved in melanocyte proliferation and differentiation, such as WNT3A, KCNMA1, KIT and HOXC8, may allow for the detection of novel low-penetration genes involved in human pigmentation and in susceptibility to skin cancer.

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CONFLICTS OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTION

BH performed the research; BH, MI-V and MP-C analysed the data; MM-G, CG-F, GR and CM-C contributed essential samples; BH, MP-C, MI-V, GR and CM-C wrote the article; GR and CM-C conceived and designed the research study; all authors reviewed the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

FIGURE S1 Comparison of minor allele frequencies between our Spanish sample and two different European populations

FIGURE S2 Enriched KEGG pathways involved in pigmentation and skin cancer risk that are targeted by miRNAs predicted to interact with highly-associated 3′UTR pigmentation SNPs

FIGURE S3 Box plot showing WNT3A expression according to SNP rs752107 genotype

FIGURE S4 Expression in different tissues of the four miRNAs predicted to interact with highly-associated 3′UTR pigmentation SNPs

TABLE S1 Classification of the Spanish individuals studied by age, sex and phenotype

TABLE S2 Minor allele frequencies in different European populations and in Spanish cases and controls

TABLE S3 Association analysis between SNPs and melanoma susceptibility in the Spanish population

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