SLC45A2 Germilne Variants in Colombian Population with Melanoma

Diana Garcia-Garay
Hospital Universitario - Centro Dermatológico Federico Lleras Acosta
David Parra-Tovar
Hospital Universitario - Centro Dermatológico Federico Lleras Acosta
Luz D Gutierrez-Castañeda (luzdgutierrezc@dermatologia.gov.co)
Hospital Universitario - Centro Dermatológico Federico Lleras Acosta

Research Article

Keywords: Melanoma, Variant, SLC45A2, population, gene

Posted Date: July 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1819800/v1

Abstract

The SLC45A2 gene, located at chromosome 5p13.2, is involved in melanin biosynthesis. Single Nucleotide Variant (SNVs) in this gene have been associated with normal variation of skin, eye and hair color in human population. Additionally, there are SNVs like L374F (C/T) and E272K (c/G) that are associated with protection against melanoma. In this project, in a survey of Bogota, Colombia people, both SNVs mentioned before were evaluated in a case and controls study. DNA samples were isolated with commercial kits, prior signing of the informed consent. The genotypification was done through RT-PCR HRM technique. We found that 19% of the individuals were classified as phototype II, 70% phototype III and 11% phototype IV. In addition, 80% of the subjects had brown eyes and dark-brown hair. Both SNVs were in Hardy-Weinberg equilibrium. Allelic frequencies were C=0.644, T=0.356 and C=0.756, G=0.244 for E272K and L374F, respectively. None of the variants analyzed had association with melanoma developing. Haplotype analysis adjusted by sex and phototype shown a higher risk of having melanoma with individuals having CG genotype, OR:2.75 (IC 95% 1.22-6.22 p=0.021). The frequencies found for the variants of SLC45A2 gene in this project is accord with many other studies done with Latin-American population, were phototype II and III are the prevalent in people.

Introduction

Type and distribution of melanin play key roles in determining human skin pigmentation, these have been associated with latitude, distribution of ultraviolet UV radiation, lifestyle, diet and metabolism, and genetic factor [1, 2]. The genetic factor plays important roles in the cascade of events leading to the biosynthesis of two types of melanin (pheomelanin and eumelanin) that have been associated with skin cancer [1, 2]. One of the genes that has been associated with the development of melanoma is *SLC45A2* gene, which is located on chromosome 5p13.2 consists of seven exons and encode a protein (a membrane-associated transporter protein or MATP). With 530 amino acids, this gene plays an important part in melanin synthesis in the melanosomes [3, 4]. The protein encode by *SLC45A2* is a member of the $K^+/Na^+/Ca^{2+}$ exchanger family of transmembrane proteins and has been proposed to regulate the Ca^{2+} concentration and pH and ionic homeostasis within the melanosome [5].

The ionic homeostasis and pH are necessary for the generation of ionic gradients that allow the translocation of tyrosinase to the melanosome. Normally and in early stages of melanosome development, acidic pH helps L-DOPA stabilization by preventing auto-oxidation, and the increase of pH by the same time of melanosome maturation optimizes tyrosinase and so melanin production. Because melanosomal pH is crucial for melanin synthesis, an acidic melanosomal pH environment could lead to the reduction or even inhibition of tyrosinase activity, altering melanin production [4, 6, 7].

Variants in the *SLC45A2* gene (p.Glu272Lys and p.Phe374Leu) has been associated with dark hair, eye pigmentation, and melanoma in diverse populations including North America, Asia, Europe, Africa, Japanese, South African European, Chinese, French, and Italians, Brazil [6, 8-10]. Pathogenic variants in this gene cause a phenotype characterized by the absence of melanin responsible for the coloration of skin, hair, eyes, and cause oculocutaneous albinism type IV (OCA4), and Albinism [8, 11, 12].

The p.Glu272Lys variant occurs in exon 3 due to a G>A transition resulting in amino acid exchanges at codon 272, and the p.Phe374Leu variant occurs due to a G>C transversion in exon 5 resulting in exchanges of amino acids at codon 374, these variants play a significant role in the functionality of the protein since it can eliminate the protein binding sites or create new binding sites [7]. However, prediction of pathogenic variants in genes can be helping to understand their roles in different mechanism into disease [13, 14]. In silico methods are used to predict whether these variants have a deleterious or neutral impact according to the analysis through the modeling of protein structures [15, 16]. In this study, we investigated the distribution of variants in *SLC45A2* gene in a sample of patients with melanoma and healthy individuals in Colombian population and computational analysis of the analyzed are performed.

Methods And Materials

Study population. All clinical, morphological, and demographic data were recollected during the obtained the informed consent for each case and control between 2018 and 2020, median age, sex and phototype were not statistically different between the groups of cases and controls. This study was approved by Research Ethics Committee of Hospital Universitario Centro Dermatologico Federico Lleras Acosta. A total of Eighty-five cases with diagnosis of cutaneous melanoma and one hundred sixty-six healthy controls subjects were included, genomic DNA was isolated from peripheral blood samples using the QIAamp® DNA Mini and Blood Mini Handbook kit according to the manufacturer's protocol and diluted to a final solution of 20 *ng/ul*.

Collecting data about risk factors for melanoma. Data on clinical characteristics (skin phototype according to the Fitzpatrick scale, age, sex, eye and hair colors, nevi count, freckles, presence of solar lentigines, family history of cancer and melanoma, history of melanoma) was collected for all cases and controls subjects. A personal interview and whole-body skin examination were performed by dermatologist and medical personal from Centro Dermatologico Federico Lleras Acosta using a standard examination report form.

Genotyping *SLC45A2* Variants. The two *SLC45A2* (p.Glu272Lys rs26722 and p.Phe374Leu rs16891982) variants were studied so that it has been associated with melanoma, 10 samples of cases and 10 samples of controls groups was performed with the Sanger method used to be as a control for after analysis using a Big Dye Terminator and BI PRISM 3130xl Genetic Analyzer (Applied Biosystems®). PCR conditions were described in the previous study and the *SCL45A2* primers (p.Glu272Lys 5- $CTGCA \top G\mathbb{C}AGCTCTGGAT$ - 3, 5- $\mathbb{C}TCAGGA\mathbb{C}C\mathbb{T}\mathbb{C}A \top GTC$ - 3 and p.Phe374Leu 5 - $GGAGAGAG \forall AGAC \top AC \forall G \forall T \forall A$ - 3, 5- $T\mathbb{C}ACAGAG \top TCTCATCTACGA$ - 3) [17]. All SNPs were genotyped using the Real-Time PCR (qPCR), and analyzed with High-Resolution Melting Analysis (HRM), all conditions were described in the previous study [17, 18].

Statistical Analysis. The main part of the statistical analysis was performed using the Stata 16 ®, all the odds ratios (OR) are reported with their 95% confidential interval (CI) and the level of significance for all test was p<0,05. The association of genetic factors and melanoma was tested with Hardy-Weinberg equilibrium by Chi-square test and OR with CI and p-<0,05 all data were assessing via logistic regressions. The analysis of genotype, haplotypes and allele frequency was determinate employed SNPStats program (http://bioinfo.iconcologia.net/SNPstats) (institute Catalá d`Oncologia, Barcelona, Spain)

association was tested according to four models (recessive, dominant, additive, and codominant genotypic) with logistic regression adjusted in both sex and familiar history of cancer.

In silico analysis of novel mutations. All characteristic of the *SLC45A2* gene were obtained from GeneCards (https://www.genecards.org), it was used to find the identifiers for *SLC45A2* Gene IDs (GC05M033981). Gene and protein sequences in the FASTA format were retrieved from Genebank (https://www.ncbi.nlm.nih.gov/genbank/) and Uniprot (https://www.uniprot.org) [19].

SMART tools (http://smart.embl-heidelberg.de) was used to explore domain architectures or find the transmembrane and cytoplasm domain of MATP protein and evaluate the position of the variants within the structure. Furthermore, ROBETTA tool (http://robetta.bakerlab.org) was used to generate PDB formats (three-dimensional structure prediction of MATP) for each variant. Subsequently, PDB formats read input files in ChimeraX software (https://www.rbvi.ucsf.edu/chimerax/) which evaluate and compare the three-dimensional structure.

On the other hand, to know what the possible impact of amino acid substitution on the biological functions and stability of proteins, a prediction by Polyphen-2 (Polymorphism Phenotyping v2 http://genetics.bwh.harvard.edu/pph2/), Pmut (http://mmb.irbbarcelona.org/PMut/), and PROVEAN (Protein Variation Effect Analyzer http://provean.jcvi.org/index.php) was done to determine or predict the pathogenicity of novel mutations, and indel has an impact of amino acid substitution on the biological function of a protein.

Results

Overall Clinical and demographics characteristics of 85 cutaneous melanoma patients and 166 healthy individual was reported in previous studies [17].

Analysis between SLC45A2 variants and phenotypic characteristics.

According to the p.Glu272Lys variant was presented in 42% in cases and 33% in controls group, and p.Phe374Leu variant was presented in 42% in cases and 48% on controls group. Regarding to the pathological characteristic – subtype of cutaneous melanoma, 17,65% cases with p.Glu272Lys had Lentigo Maligna Melanoma (LMM), 17.56% had an IV Clark level, 9.41% had a Breslow scale >4,0mm and the most common location was in head and neck with 21,18%. On the other hand, 16,47% cases with p.Phe374Leu variant had LMM, 20% had an IV Clark level, 11,76% had a Breslow scale >4,0mm and 18,82% was location in head and neck.

About clinical characteristic of the cases and controls group with p.Glu272Lys variant, 30.59% in cases and 24% in controls were subjects with phototype II, and 23,35% in cases and 25,30% in controls had black or dark brown in eye color, 30,59% in cases and 28,92% in controls had black nor dark brown hair colors. Regarding the p.Phe374Leu variant, the most common phototype in cases and controls were phototype II with 36,47% and 37,95%, respectively. In the eyes and hair colors, the most frequency was black or dark brown in both, cases and controls. Finally, <50 nevus was presented in all individuals (Table 1) (Supplementary table S1 Figure 1 and 2).

Table 1. Clinical and pathological feature of cases and controls group.

FEATURE	CASES 85				CONTROLS 166				CASES 85				CONTROLS 166	
	p.Glu272Lys				p.Glu272Lys				p.Phe374Leu				p.Phe374Leu	
	Presence	%	Absence	%	Presence	%	Absence	%	Presence	%	Absence	%	Presence	%
AGE														
MEAN	59		59		58		58		60		58		58	
SEX														
WOMEN	21	25%	25	29%	29	17%	60	36%	20	24%	26	31%	44	27%
MEN	15	18%	24	28%	27	16%	49	30%	18	21%	21	25%	37	22%
MELANOMA SUBTYPE														
SUPERFICIAL SPREAD	8	9%	12	14%	-	-	-	-	8	9%	12	14%	-	-
NODULAR	4	5%	9	11%	-	-	-	-	5	6%	8	9%	-	-
ACRAL LENTIGINOUS	9	11%	12	14%	-	-	-	-	11	13%	10	12%	-	-
LENTIGO MALIGNA	15	18%	16	19%	-	-	-	-	14	16%	17	20%	-	-
CLARK LEVEL														
NEGATIVE	17	20%	16	19%	-	-	-	-	17	20%	16	19%	-	-
I	2	2%	0	0%	-	-	-	-	1	1%	1	1%	-	-
II	1	1%	7	8%	-	-	-	-	1	1%	7	8%	-	-
III	1	1%	1	1%	-	-	-	-	1	1%	1	1%	-	-
IV	15	18%	20	24%	-	-	-	-	17	20%	18	21%	-	-
V	0	0%	5	6%	-	-	-	-	1	1%	4	5%	-	-
BRESLOW SCALE														
NON REPORTED	17	20%	15	18%	-	-	-	-	16	19%	16	19%	-	-
≤ 1,0 mm	4	5%	7	8%	-	-	-	-	4	5%	7	8%	-	-
>1,0 - 2,0 mm	1	1%	8	9%	-	-	-	-	2	2%	7	8%	-	-
>2,0 - 4,0 mm	6	7%	6	7%	-	-	-	-	6	7%	6	7%	-	-
>4,0 mm	8	9%	13	15%	-	-	-	-	10	12%	11	13%	-	-
LOCATION														
TRUNK	5	6%	7	8%	-	-	-	-	7	8%	5	6%	-	-
HEAD AND NECK	18	21%	19	22%	-	-	-	-	16	19%	21	25%	-	-
UPPER EXTREMITIES	2	2%	3	4%	-	-	-	-	0	0%	5	6%	-	-
LOWER EXTREMITIES	2	2%	8	9%	-	-	-	-	4	5%	6	7%	-	-
HANDS AND FEET	9	11%	12	14%	-	-	-	-	11	13%	10	12%	-	-
PHOTOTYPE														
2	4	5%	12	14%	8	5%	23	14%	4	5%	12	14%	10	6%
3	26	31%	33	39%	40	24%	76	46%	31	36%	28	33%	63	38%
4	6	7%	4	5%	8	5%	9	5%	3	4%	7	8%	8	5%
EYE COLOR														
BLACK OR DARK BROWN	19	22%	25	29%	42	25%	85	51%	20	24%	24	28%	63	38%
LIGHT BROWN	12	14%	18	21%	11	7%	16	10%	11	13%	19	22%	13	8%

GREEN	4	5%	5	6%	1	1%	8	5%	5	6%	4	5%	1	1%
BLUE	1	1%	1	1%	2	1%	5	3%	2	2%	0	0%	4	2%
HAIR COLOR														
BLACK OR DARK BROWN	26	31%	29	34%	48	29%	87	52%	30	35%	25	29%	69	42%
LIGHT BROWN	10	12%	19	22%	7	4%	21	13%	8	9%	21	25%	12	7%
RED OR BLOND	0	0%	1	1%	1	1%	1	1%	0	0%	1	1%	0	0%
FAMILY HISTORY OF CANCER														
YES	20	24%	29	34%	28	17%	54	33%	25	29%	24	28%	41	25%
NO	16	19%	20	24%	28	17%	55	33%	13	15%	23	27%	40	24%
NEVUS														
≤ 50	33	39%	38	45%	54	33%	106	64%	33	39%	38	45%	81	49%
50 - 100	2	2%	9	11%	2	1%	3	2%	4	5%	7	8%	0	0%
>100	1	1%	2	2%	0	0%	0	0%	1	1%	2	2%	0	0%

SLC45A2 Variants analysis.

Within the analysis of allele and genotypic results for the rs26722 and rs16891982 variants in *SLC45A2* gene it was shown that the C allele of the rs26722 variant was associated as a protective factor for melanoma in the analyzed population OR=0,656 (CI 95% 0,44 - 0,96) p=0,029. In contrast, the G allele of the rs16891982 variant was associated with the development of melanoma in the analyzed population OR=2,447 (CI 95% 1,488 – 4,025) p=0,0003 (Table 2). The rs26722 and rs16891982 variants were presented in 58% and 45% for cases and 70% and 49% for controls groups, respectively. According to the types of genetic models' analysis (Dominant, Recessive, Codominant, Overdominant and Log-additive) adjusted by sex and family history of cancer were used to determined association between variants and develop melanoma but did not display any statistical differences in case and control groups (Table 3 and supplement table 1).

Table 2. Allele and genotype distribution of SLC45A2 variants in cases and control groups.

Genotype/Allele	Cases n=85 (%)	Control n= 166 (%)	OR	(95% CI)	p-Value	⊠2
SLC45A2 **(rs26722 p.E272K)**						
T/T	36 (42)	50 (30)	1	Reference		
T/C	0 (0.0)	6 (4)	0,144	(0,008-2,594)	0,128	2,313
C/C	49 (58)	109 (66)	0,711	(0,416-1,217)	0,213	1,548
C	**98 (58)**	**224 (67)**	**0,656**	**(0,44-0,96)**	**0,029*** **	**4,716**
SLC45A2 **(rs16891982 p.L374F)**						
C/C	47 (55)	84 (51)	1	Reference		
G/C	37 (44)	79 (48)	0,848	(0,501-1,43)	0,541	0,373
G/G	1 (1)	2 (1)	0,976	(0,08-10,92)	0,984	0,0004
G	**39 (23)**	**83 (25)**	**2,447**	**(1,488-4,025)**	**0,0003*** **	**12,948**

Table 3. Genetic models analysis for rs26722 and rs16891982 variants in SCL45A2 gene in case and control groups

*SCL45A2*_ rs26722 p.E272K association with response group (n=250, adjusted by sex+family)					
Model	**Genotype**	**group=Ca**	**group=Co**	**OR (95% CI)**	**P-value**
Codominant	C/C	49 (57.6%)	109 (66.1%)	1.00	0.018
	C/T	0 (0%)	6 (3.6%)	NA (0.00-NA)	
	T/T	36 (42.4%)	50 (30.3%)	0.64 (0.34-1.21)	
Dominant	C/C	49 (57.6%)	109 (66.1%)	1.00	0.36
	C/T-T/T	36 (42.4%)	56 (33.9%)	0.75 (0.40-1.40)	
Recessive	C/C-C/T	49 (57.6%)	115 (69.7%)	1.00	0.12
	T/T	36 (42.4%)	50 (30.3%)	0.61 (0.32-1.14)	
Overdominant	C/C-T/T	85 (100%)	159 (96.4%)	1.00	0.013
	C/T	0 (0%)	6 (3.6%)	NA (0.00-NA)	
Log-additive	—	—	—	0.82 (0.59-1.12)	0.22
*SCL45A2*_ rs16891982 p.L374F association with response group (n=250, adjusted by sex+family)					
Model	**Genotype**	**group=Ca**	**group=Co**	**OR (95% CI)**	**P-value**
Codominant	C/C	47 (55.3%)	84 (50.9%)	1.00	0.78
	C/G	37 (43.5%)	79 (47.9%)	1.24 (0.67-2.30)	
	G/G	1 (1.2%)	2 (1.2%)	1.36 (0.08-23.59)	
Dominant	C/C	47 (55.3%)	84 (50.9%)	1.00	0.48
	C/G-G/G	38 (44.7%)	81 (49.1%)	1.24 (0.67-2.29)	
Recessive	C/C-C/G	84 (98.8%)	163 (98.8%)	1.00	0.88
	G/G	1 (1.2%)	2 (1.2%)	1.25 (0.07-21.51)	
Overdominant	C/C-G/G	48 (56.5%)	86 (52.1%)	1.00	0.5
	C/G	37 (43.5%)	79 (47.9%)	1.23 (0.67-2.28)	
Log-additive	—	—	—	1.23 (0.69-2.20)	0.48

Haplotype analysis

The analysis of the haplotypes adjusted for sex and phototype showing that CG haplotype is a risk factor for melanoma, OR= 2.71 (CI 95% 1.20 -6.13), *p= 0,018* (Table 4)

Table 4. Haplotype association adjusted by sex and phototype.

p.E272K	p.L374F	Frequency	OR (95% CI)	*P-value*
C	C	0.5372	1	—
T	C	0.2182	1.09 (0.67 – 1.76)	0.73
T	G	0.1407	0.74 (0.39 – 1.40)	0.35
C	**G**	**0.104**	**2.71 (1.20 - 6.13)**	**0.018***

Analysis of clinical factors and genetic factors with melanoma.

Clinical factors were grouped individually into two groups (phototype I-II vs. III-IV, eyes color light vs. black, hair color red-blond-brown vs. dark brown-black, and nevus count <50 vs >50) for analysis between clinical factors and genetic factors (rs26722 and rs16891982). We calculated the ORs resulting from combining these factors and the *SLC45A2* variants, we found that p.Phe374Leu variant is associated as a protective factor in the population with phototype III-IV and dark brown and black hair, OR=0,380 – p=0,004 and OR=0,459 – p=0,011, respectively (Table 5). We also carried out a map of relationships between the variables of phototype, genotype's, cases and controls group, and the sex of the analyzed population, and we found a strong relationship between the variables groups with phototype, the variants p.Phe374Leu and the sex of the patients (Figure 1).

Table 5. Association between clinical and genetic factors.

rs26722 p.Glu272Lys			
Clinical factor	OR	CI 95%	p-Value
Phototype II vs. III-IV	0,508	0,24-1,35	0,059
Eyes color light vs. Black	1,006	0,58-1,73	0,980
Hair color red-blond-brown vs. dark brown-black	0,671	0,35-1,25	0,210
Nevus count <50 vs >50	0,591	0,20-1,69	0,324
rs16891982 p.Phe374Leu			
Phototype II vs. III-IV	0,380	0,19-0,75	0,004*
Eyes color light vs. Black	0,75	0,44-1,27	0,285
Hair color red-blond-brown vs. dark brown-black	0,459	0,25-0,84	0,011*
Nevus count <50 vs >50	0,366	0,12-1,05	0,053

Modeling of MATP protein structure and validation.

The search for information the gene and protein sequence in FASTA format was downloaded from the Genebank and Uniprot databases, then the intramembrane and cytoplasmic domains were obtained using SMART (Figure 2). After, Robetta was used to obtain the PDB format and perform the three-dimensional prediction of the protein and include the variants analyzed as shown in figure 3, this protein structure was obtained by the ChimeraX software (Figure 3). Once association of the variants with risk and protective factors for melanoma in the analyzed population was known. We evidenced structural changes in the modeling of the protein in three-dimensional prediction. We wanted to know if these variants generated a pathogenic effect and their impact on the biological function of the protein. Therefore, we see that the variant p.Phe374Leu according to the predictions has a probably pathogenic effect on the function of the protein, while the change of this amino acid in the variant p.Glu272Lys does not present any pathogenic effect on the function of the protein the change of this amino acid (Table 6).

Table 6. Prediction of the probably effect of the p.Glu272Lys and p.Phe374Lue variants in the function of the MATP protein using Provean, Polyphen and Pmut. .

Variant	Score PROVEAN	Prediction (cutoff= -2.5)	Score Polyphen	Prediction	Score Pmut	Prediction
p.L374F	-2.675	Deleterious	0,991	Probably damaging	0,42	Neutral
p.E272K	-1.095	Neutral	0,003	Benign	0,19	Neutral

Discussion

It has been widely known that etiological factors associated with risk of skin cancer include skin phenotype, geographic location, and sun-exposure. Skin pigmentation is one of the characteristics most associated with the susceptibility to melanoma, since the presence of pheomelanin (light skin color) is determined as a risk factor for the development of this disease, while the presence of eumelanin has been accepted as a protective factor [20]. This trait is determined by the interaction of the products of several genes [21]. Variants in these genes together with environmental factors can modulate the population risk of developing melanoma. The study of these polymorphic variants in different populations allows us to understand the adaptive genetic characteristics of a population to a certain geographic region and to establish a correlation between the genotype and phenotype of the individual with the aim of identify predictors of susceptibility to a disease. In this study, we evaluated the frequency of two SNPs in the *SCL45A2* gene in a population that lives in equatorial region and is characterized by a mixture of African, European, and indigenous ancestry.

SCL45A2 gene variants have been initially associated with susceptibility to melanoma, due it shows different population frequency and have been associated with dark hair, dark skin, and dark eye pigmentation [22, 23]. In this study, the p.Glu272Lys (c.814C>T; rs26722) and p.Phe374Leu (c.1122C>G; rs16891982) variants in the *SCL45A2* gene were evaluated in 85 cases and 166 controls. The presence of allele C of the rs26722 variant shown to be a protective factor for the development of melanoma in this population (Table 2). Genotype analysis showed no association with any of the phenotypic traits associated with susceptibility to melanoma. On the contrary, the presence of allele G of the rs16891982 variant show to be a risk factor to melanoma. Furthemore, when the genotype and its association with development adjusted for sex, phototype and familiar history are analyzed for the two SNPs, no association was found in any model of inheritance.

Fernández L, *et al* showed that p.Phe374Leu variant is a factor risk to melanoma in subjects from Spaniard origin (Madrid) (OR, 0.41; 95% CI, 0.24-0.7) [12]. Likewise, a meta-analysis of studies from French, Italian and Spaniards populations showed that the p.Phe374Leu variant retains a protective factor when adjusted for other genetic and phenotypic factors [2, 3]. Interestingly, the presence of the minor allele in these southern European populations is related to dark skin, hair and eye color [2]. In our study we found that phototype II individuals with this variant have a protective factor (*p=0.004*) against those having phototype III and IV, which correlates with studies in individuals of European ancestry.

The p.Phe374 allele is fixed in European populations, while p.Leu374 allele is fixed in African populations, this characteristic is wrongly associated with skin pigmentation [24]. The presence of Phenylalanine at position 374 of the protein encoded by *SLC45A2* (S45A2: Membrane-associated transporter protein) makes it less stable than the p.Leu374 variant and leads to rapid degradation, resulting in reduced levels of the protein, and therefore it negatively affects tyrosinase activity, leads to less production of eumelanin and generates a lighter pigmentation [25]. For this reason, the presence of p.Phe374 is a risk factor for melanoma in populations with light phototypes. Likewise, as shown in the prediction analysis, the presence of the 374Phe variant has a probable pathogenic effect, however, a negative selective pressure has been shown in individuals in Northern Europe for the ancestral G allele [26], as well as a gradient increase in the frequency of the same allele (G; 374L) from the North to the South of Spain according to increase the UV radiation [24]. This indicates that skin depigmentation was an evolutionary adaptive process, and that eumelanin production is a protective factor for the development of skin cancer in individuals living in regions with higher UV radiation.

The present study analyzed a sample of individuals mostly living in a geographical region above the Andes Mountain range at 2600m above sea level located in the tropic region of South America, which is characterized by a high UV radiation index [27, 28]. Additionally, this region is characterized by a low incidence of melanoma compared to countries with a lower UV radiation index in Europe [28]. The predominant phototypes in the population analyzed were phototype III and IV and light to dark brown hair and eye color. According to genetic ancestry studies of this population (Cundi-boyacense), it has been determined that the genetic background is represented by 0.51% to 4.2% African, 55% European and 36% to 40.8% Native American [29, 30]. It is possible that in this population the enrichment of African ancestral alleles (G; 374L) that participate in a higher production of eumelanin are related to the protection for melanoma and that together with other genetic and environmental factors determine the incidence of this disease.

Despite the high European contribution in this region, the frequency of the G allele (rs16891982) in the sample analyzed was 23% and 25% in cases and controls respectively, indicating the genetic admixture of the population. This frequency is similar to that found in a population from the northern region of the state of Sao Paulo, Brazil (35.67%), but much higher than that found in European regions (3.8%; Germany, 2.9%; northern Europe and 4% southern Europe), and lower than that found in the African population (94.1%) [13, 24, 31]. While the behavior of the T allele at SNP c.814C>T; (rs26722) shows a different behavior. The frequency for the T allele was 42% and 23% in cases and controls respectively, similar to the frequency found in the Japanese population (38%). In the northern Brazilian population, the frequency of this allele was 9%, very similar to the frequencies found in the European and African populations (3% and 5% respectively) [13]. In analyzes carried out on the European population, the presence of the T allele is a protective factor for melanoma [24], while the results of this study show that the C allele is the protective factor. Thus, we also found that even though the genotype analysis does not show an association with melanoma, the presence of the CG haplotype is a risk factor when adjusted for phototype and age.

The difference in population frequencies in Latin America reported for the analysis of variants in the *SCL45A2* gene are consistent with the results of the CANDELA study which shows that the ancestry of the Latin American regions is heterogeneous given the different migration patterns [32]. Likewise, in Colombia, it has been shown that the different geographical regions present differences in the contribution of African, European and Native American ancestry [29]. This heterogeneity and the results found in this study suggest that studies should be carried out to determine allele frequencies by stratifying the population by region and considering phenotypic characteristics. Thus considering the results of the relationship analysis done in this study were the phototype II is more closely related to individuals diagnosed with melanoma, so knowledge of the relationship between genotype and phenotypic characteristics of individuals can help determine susceptibility to melanoma.

In conclusion, the allelic contribution of the c.814C>T and c.1122C>G variants of the *SCL45A2* gene found in this study, it is necessary to expand the sample size and study other variants related to the melanogenesis pathway and their contribution to melanoma susceptibility in this population.

Declarations

Conflict of interest statement: no author declares conflict of interest.

Funding Statement: Hospital Universitario - Centro Dermatológico Federico Lleras Acosta E.S.E, DC, Colombia. Grant numbers [1DSI02-6AE].

Author's contributions

Diana Katherinne Garcia Garay: Data collection, or analysis and interpretation of data; writing of the manuscript or critical review of important intellectual content; data collection, analysis, and interpretation; critical review of the literature.

David Tovar-Parra: Data collection, or analysis and interpretation of data; statistical analysis; writing of the manuscript or critical review of important intellectual content; data collection, analysis, and interpretation; critical review of the literature.

Luz D Gutierrez-Castañeda: The study concept and design; data collection, or analysis and interpretation of data; statistical analysis; writing of the manuscript or critical review of important intellectual content; data collection, analysis, and interpretation; critical review of the literature, final approval of the final version of the manuscript.

References

1. Wang Y. Association of pigmentation related-genes polymorphisms and geographic environmental variables in the Chinese population. Hereditas. 2021;158(1):24.
2. Ibarrola-Villava M, Hu H-H, Guedj M, Fernandez LP, Descamps V, Basset-Seguin N, et al. MC1R, SLC45A2 and TYR genetic variants involved in melanoma susceptibility in southern European populations: results from a meta-analysis. European journal of cancer. 2012;48(14):2183-91.

3. Guedj M, Bourillon A, Combadières C, Rodero M, Dieudé P, Descamps V, et al. Variants of the MATP/SLC45A2 gene are protective for melanoma in the French population. Human mutation. 2008;29(9):1154-60.
4. Bin BH, Bhin J, Yang SH, Shin M, Nam YJ, Choi DH, et al. Membrane-Associated Transporter Protein (MATP) Regulates Melanosomal pH and Influences Tyrosinase Activity. PLoS One. 2015;10(6):e0129273.
5. Andrade ES, Fracasso NC, Júnior PSS, Simões AL, Mendes-Junior CT. Associations of OCA2-HERC2 SNPs and haplotypes with human pigmentation characteristics in the Brazilian population. Legal Medicine. 2017;24:78-83.
6. Fracasso N, Andrade E, Andrade C, Zanão L, Silva M, Marano L, et al. Association of SNPs from the SLC45A2 gene with human pigmentation traits in Brazil. Forensic Science International: Genetics Supplement Series. 2013;4(1):e342-e3.
7. Reinders A, Ward JM. Investigating polymorphisms in membrane-associated transporter protein SLC45A2, using sucrose transporters as a model. Mol Med Rep. 2015;12(1):1393-8.
8. Lucotte G, Mercier G, Diéterlen F, Yuasa I. A decreasing gradient of 374F allele frequencies in the skin pigmentation gene SLC45A2, from the north of West Europe to North Africa. Biochemical genetics. 2010;48(1-2):26-33.
9. Branicki W, Brudnik U, Draus-Barini J, Kupiec T, Wojas-Pelc A. Association of the SLC45A2 gene with physiological human hair colour variation. Journal of human genetics. 2008;53(11-12):966.
10. Vierkötter A, Krämer U, Sugiri D, Morita A, Yamamoto A, Kaneko N, et al. Development of lentigines in German and Japanese women correlates with variants in the SLC45A2 gene. J Invest Dermatol. 2012;132(3 Pt 1):733-6.
11. Ali MH, Vajzovic L. Albinism. In: Cynthia A. Toth SSO, editor. Handbook of Pediatric Retinal OCT and the Eye-Brain Connection. Elsevier2019. p. 115-8.
12. Fernandez L, Milne R, Pita G, Aviles J, Lazaro P, Benitez J, et al. SLC45A2: a novel malignant melanoma-associated gene. Human mutation. 2008;29(9):1161-7.
13. Yuasa I, Umetsu K, Harihara S, Kido A, Miyoshi A, Saitou N, et al. Distribution of the F374 allele of the SLC45A2 (MATP) gene and founder-haplotype analysis. Annals of human genetics. 2006;70(6):802-11.
14. Tóth L, Fábos B, Farkas K, Sulák A, Tripolszki K, Széll M, et al. Identification of two novel mutations in the SLC45A2 gene in a Hungarian pedigree affected by unusual OCA type 4. BMC Med Genet. 2017;18(1):27.
15. Bibi N, Ullah A, Darwesh L, Khan W, Khan T, Ullah K, et al. Identification and Computational Analysis of Novel. Front Genet. 2020;11:749.
16. Miller PJ, Duraisamy S, Newell JA, Chan PA, Tie MM, Rogers AE, et al. Classifying variants of CDKN2A using computational and laboratory studies. Hum Mutat. 2011;32(8):900-11.
17. Tovar-Parra JD, Gutiérrez-Castañeda LD, Gil-Quiñones SR, Nova JA, Pulido L. CDKN2A Polymorphism in Melanoma Patients in Colombian Population: A Case-Control Study. Biomed Res Int. 2020;2020:7458917.
18. Tovar-Parra D, Gil-Quiñones SR, Nova J, Gutiérrez-Castañeda LD. 3'UTR-CDKN2A and CDK4 Germline Variants Are Associated With Susceptibility to Cutaneous Melanoma. In Vivo. 2021;35(3):1529-36.
19. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics. 2016;54:1.30.1-1..3.
20. Volkovova K, Bilanicova D, Bartonova A, Letašiová S, Dusinska M. Associations between environmental factors and incidence of cutaneous melanoma. Review. Environmental Health. 2012;11(1):S12.
21. Deng L, Xu S. Adaptation of human skin color in various populations. Hereditas. 2018;155(1):1-12.
22. Graf J, Hodgson R, van Daal A. Single nucleotide polymorphisms in the MATP gene are associated with normal human pigmentation variation. Hum Mutat. 2005;25(3):278-84.
23. Han J, Kraft P, Nan H, Guo Q, Chen C, Qureshi A, et al. A genome-wide association study identifies novel alleles associated with hair color and skin pigmentation. PLoS genetics. 2008;4(5):e1000074.
24. López S, García Ó, Yurrebaso I, Flores C, Acosta-Herrera M, Chen H, et al. The interplay between natural selection and susceptibility to melanoma on allele 374F of SLC45A2 gene in a South European population. PloS one. 2014;9(8):e104367.
25. Le L, Escobar IE, Ho T, Lefkovith AJ, Latteri E, Haltaufderhyde KD, et al. SLC45A2 protein stability and regulation of melanosome pH determine melanocyte pigmentation. Molecular biology of the cell. 2020;31(24):2687-702.
26. Soejima M, Tachida H, Ishida T, Sano A, Koda Y. Evidence for recent positive selection at the human AIM1 locus in a European population. Molecular biology and evolution. 2006;23(1):179-88.
27. González J. ULTRAVIOLET INDEX IN BOGOTA DURING THE NIÑA PHENOMENON. 2012.
28. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021;71(3):209-49.
29. Ossa H, Aquino J, Pereira R, Ibarra A, Ossa RH, Pérez LA, et al. Outlining the ancestry landscape of Colombian admixed populations. PloS one. 2016;11(10):e0164414.
30. Sanchez I, Mendoza L, Rincón AL, Aguirre DP, Builes JJ. Ancestry evaluation in a population sample of the Tunja city, Department of Boyacá-Colombia. Forensic Science International: Genetics Supplement Series. 2019;7(1):695-7.
31. de Aguiar Fracasso NC, de Andrade ES, Wiezel CEV, Andrade CCF, Zanão LR, da Silva MS, et al. Haplotypes from the SLC45A2 gene are associated with the presence of freckles and eye, hair and skin pigmentation in Brazil. Legal Medicine. 2017;25:43-51.

32. Chacón-Duque J-C, Adhikari K, Fuentes-Guajardo M, Mendoza-Revilla J, Acuña-Alonzo V, Barquera R, et al. Latin Americans show wide-spread Converso ancestry and imprint of local Native ancestry on physical appearance. Nature communications. 2018;9(1):1-13.

Figures

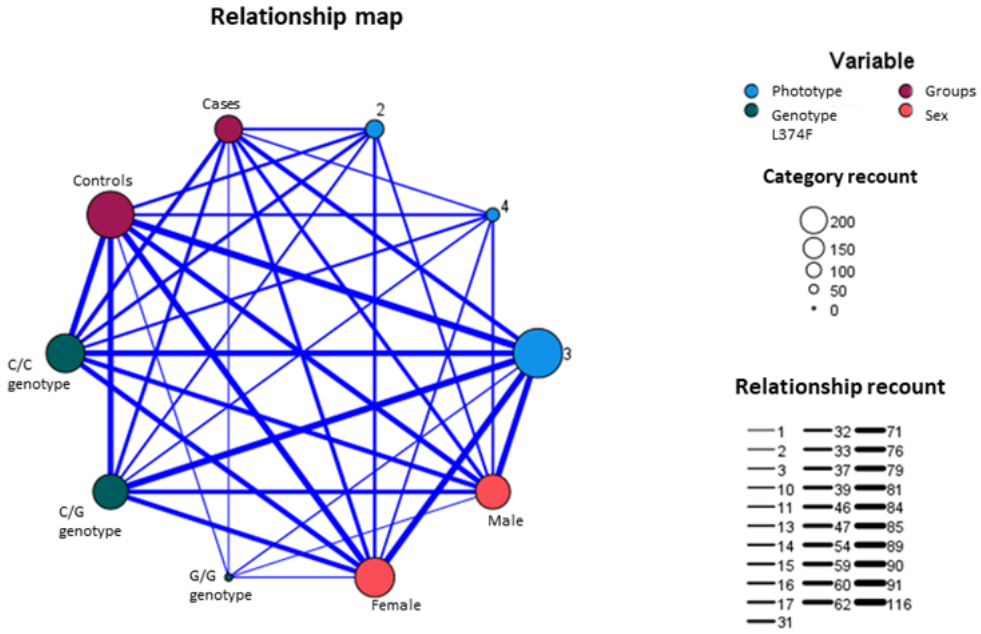

Figure 1

Relationship diagram between phototype, sex, groups and p.Phe374Leu. Category recount circle's sizes represent the rounded value for members of each category. Relationship recount line's thicknesses represents the reounded value for the member of a catgeory that are related to another one. Blue circles represent the phototype variable. Red circles represent groups (cases and controls). Peach circles represent sex (male and female). And green circles, represent Genotype for rs16891982 variant.

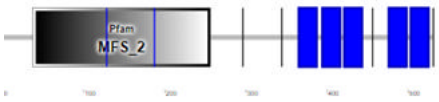

Figure 2

Transmembrane and cytoplasmic of the protein sequence using SMART. In gray box, MFS_2 domain, (Major Facilitator Superfamily type 2) associated with sugar transport proteins. Blue lines within gray box and black lines, representation of introns. Blue boxes, representation of transmembrane regions.

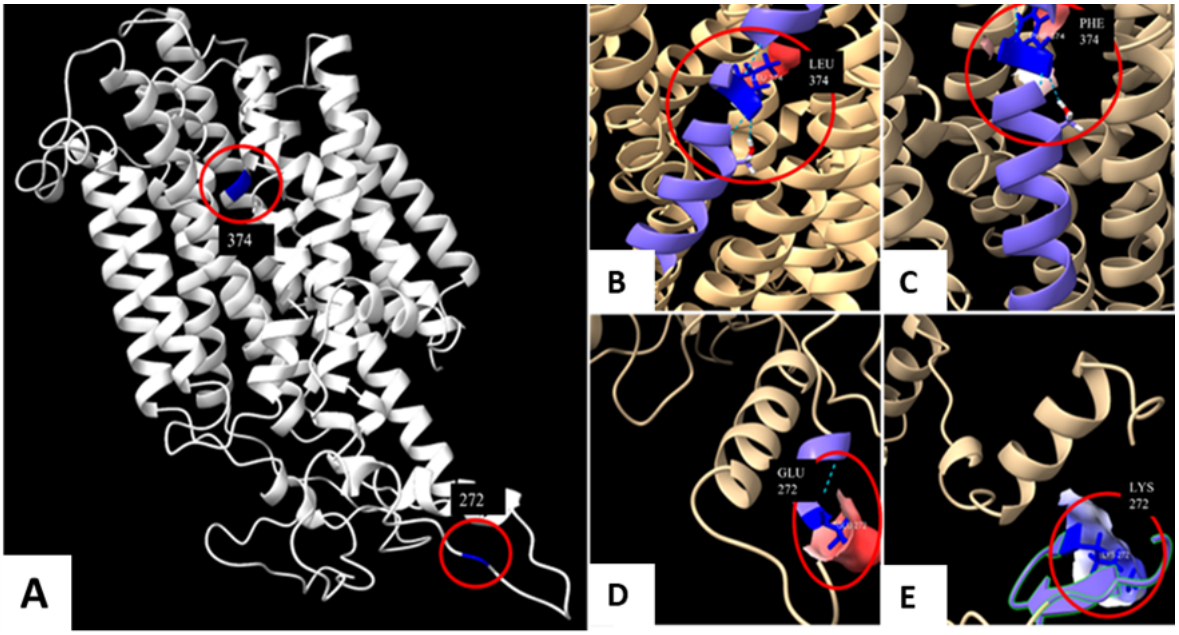

Figure 3

Structure of MATP protein prediction in ChimeraX Software. This structure was predicted using the PDB formats obtained by ROBETTA tool. A. Front view of the protein, positions 272 and 374 are marked with blue color. B and C. Front view of the L374 and F374 variants, respectively. D and E show the structure of each amino acid, the hydrogen bonds it forms (dotted lines) as well as the simulation of the surface based on the electrostatic Coulomb potential (it varies from red to blue, with red being a residue with a higher potential negative and blue with a more positive potential).