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High *SLC7A11* expression in normal skin of melanoma patients

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ABSTRACT

Background: Melanoma is one of the highest metastatic cancers and its incidence is rapidly increasing. A great effort has been devoted to determine gene mutations and expression profiles in melanoma cells, but less attention has been given to the possible influence of melanin synthesis in melanocytes on melanomagenesis. *SLC7A11* encodes the cystine/glutamate antiporter xCT and its expression increases the antioxidant capacity of cells by providing cysteine that may be used for glutathione (GSH) synthesis. Melanocytes, however, can also use cysteine for pheomelanin synthesis and pigmentation. Therefore, pheomelanin synthesis may lead to chronic oxidative stress. Possible consequences of this for melanomagenesis have never been explored.
Methods: We quantified the expression of *SLC7A11* and other genes that are involved in the synthesis of pheomelanin but do not regulate the transport of cysteine from the extracellular medium to the cytosol (*CTNS*, *MC1R*, *ASIP* and *SLC45A2*) in non-tumorous skin of 45 patients of cutaneous melanoma and 50 healthy individuals. We controlled for the effects of Fitzpatrick skin type, age, gender, body mass, frequency of sun exposure and sunburns and number of melanocytic nevi, as well as for the intrinsic antioxidant capacity as given by the expression of the gene *NFE2L2*.
Results: The expression of *SLC7A11*, but not of the other genes, was significantly higher in melanoma patients than in healthy individuals. This was independent of phenotypic factors and antioxidant capacity, thus supporting an effect of pheomelanin-induced oxidative stress on melanomagenesis.
Conclusion: Our findings indicate that *SLC7A11* downregulation in normal epidermal melanocytes may represent a preventive treatment against melanoma.

1. Introduction

Melanoma cells are one of the highest metastatic cancer cells [1], and the incidence of invasive melanoma has risen steadily in the last two decades worldwide [2]. A significant effort is being devoted to the identification of mutations in genes [3–5] and gene expression profiles [6,7] associated to melanoma progression. Most of these studies have focused on tumor cells either *in vitro* or *in vivo*, but less attention has been given to the physiology of normal melanocytes that can potentially become melanoma cells. The fact that melanocytes synthesize melanin pigments has been treated with relative little consideration, though the amount of melanins synthesized has been shown to affect the mechanical properties of melanocytes and their capacity to metastasize [8]. The melanogenic activity of melanocytes can hardly be inhibited, but the chemistry of the melanins that are synthesized may be partly modified, although genes regulating melanin synthesis have barely been investigated in relation to melanoma beyond the melanocortin 1 receptor (*MC1R*) gene [9]. Recent findings, however, indicate that melanin chemistry fulfills an essential role in melanomagenesis, opening new avenues for melanoma treatment. In particular, the synthesis pathway of the sulphurated form of melanin, termed pheomelanin, has been shown to be involved in melanoma progression by a mechanism of reactive oxygen species (ROS)-mediated stress causing DNA damage that is independent of exposure to ultraviolet (UV) light [10]. In the past few decades, Prota et al. had already highligthed the association between pheomelanin and melanomagenesis [11].

Oxidative stress is probably generated during the pheomelanin synthesis pathway in melanocytes because the sulfhydryl group of cysteine is incorporated into the pigment structure, which can reduce the availability of cysteine to form the antioxidant glutathione (GSH) and

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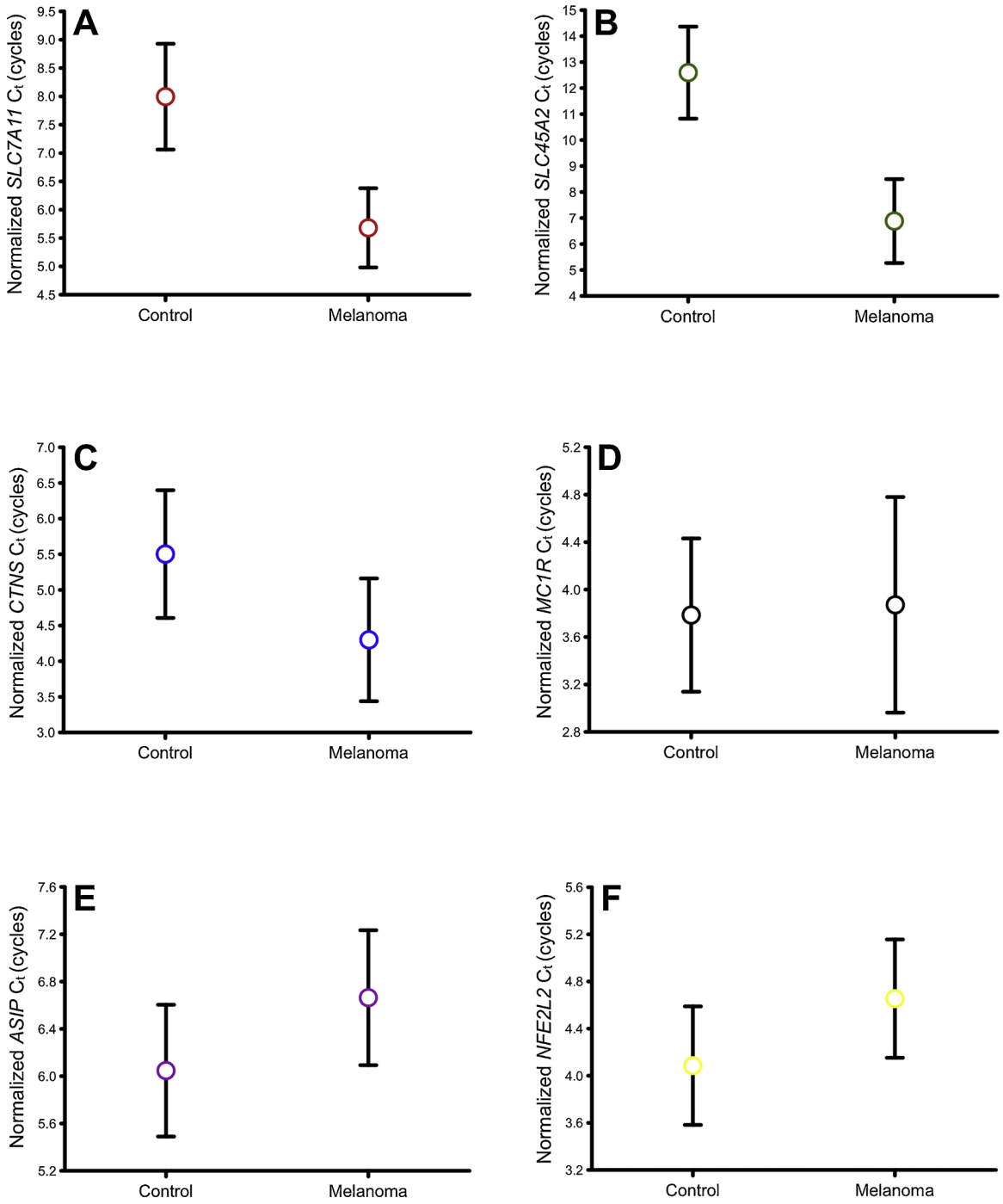

Fig. 1. Expression levels of the genes *SLC7A11* (A), *SLC45A2* (B), *CTNS* (C), *MC1R* (D), *ASIP* (E) and *NFE2L2* (F) in normal skin. Mean ± sem values corresponding to healthy individuals (control) and patients of cutaneous melanoma are shown. Expression was measured by means of mRNA amounts quantified by cycle threshold (C_t) levels and normalized by substraction of C_t values corresponding to the gene *ACTB* in the same samples (ΔC_t). Lower C_t values indicate higher mRNA levels and higher gene expression levels. Differences between controls and melanoma patients were statistically significant only in A and B.

thus produce a situation of chronic oxidative stress when pheomelanin synthesis is abundant and constant [12,13]. Thus, intramelanocytic cysteine can enter the pheomelanin synthesis pathway instead of the GSH synthesis pathway, and as pheomelanin is transferred to keratinocytes and associated epidermal structures such as hair [14], cysteine used for pheomelanin synthesis cannot be recovered for physiological use as an antioxidant.

Pheomelanin-mediated oxidative stress may be key for understanding melanomagenesis because it leads to a novel perspective of melanocytes: these are the only cells that synthesize pheomelanin, making that part of cellular cysteine is used for pheomelanin synthesis instead of GSH synthesis. This may limit the antioxidant capacity of melanocytes. The recognition of this characteristic feature of melanocytes may help to understand the outcome of melanoma. In this regard, the gene *SLC7A11* (solute carrier family 7 member 11), which encodes the cystine/glutamate antiporter xCT (system x_c^-) [15], is highly expressed in cancer cells because of its regulatory role of cystine uptake and incorporation into GSH [16]. *SLC7A11* thus enhances the antioxidant capacity of cells, and its inhibition, which can be mediated by p53, induces ferroptosis upon oxidative stress [17]. This explains why *SLC7A11* is highly expressed in several cancer types such as breast [18], bladder [19] and glioma [20]. If *SLC7A11* is expressed in normal

melanocytes, the synthesis of pheomelanin by these cells may cause chronic oxidative stress, however. Thus, melanoma progression may not only be affected by *SLC7A11* expression in melanoma cells, but also by *SLC7A11* expression in normal melanocytes. This may cause oxidative stress at the level of organism that makes melanocytes prone to DNA damage and to become, therefore, melanoma cells [7]. This possibility has never been explored, but pheomelanin is synthesized by epidermal melanocytes in all skin types [21], meaning that pheomelanin-mediated oxidative stress can potentially occur in all pigmentation phenotypes.

Here we show that *SLC7A11* expression in normal skin (*i.e.*, not affected by melanoma lesions) is significantly higher in cutaneous melanoma patients than in healthy individuals, pointing to a previously unsuspected marker of melanoma risk. This is independent of several phenotypic and behavioral factors known to affect the risk of melanoma, notably Fitzpatrick skin type, but also age, gender, body mass, number of melanocytic nevi, frequency of sun exposure and sunburn frequency [22–27]. This is also independent of the intrinsic antioxidant capacity as given by the expression of the gene *NFE2L2*, which encodes the transcription factor NRF2, the master regulator of the cellular antioxidant response [28]. Additionally, we investigated other genes involved in pheomelanin synthesis, but no differential expression between melanoma patients and controls was found. These genes are *CTNS*, which codes for a cystine/H^+ symporter that exports cystine out of melanosomes [29]; *MC1R*, which codes for the melanocortin 1 receptor in the membrane of melanocytes to which melanocortins bind and change intracellular cAMP levels, and *ASIP*, which codes for the antagonist agouti signaling protein [30]; and *SLC45A2* (solute carrier family 45 member 2), whose function is not clear but has also been suggested to code for a transporter of cysteine into melanosomes [31]. Our results suggest that a high cysteine transport activity carried out by *SLC7A11* in normal skin explains the incidence of melanoma.

2. Results

Our study comprised 45 patients of cutaneous melanoma and 50 healthy individuals that served as controls. We could measure mRNA levels in normal skin in 53 individuals for *SLC7A11* (23 melanoma and 30 controls), 45 individuals for *CTNS* (19 melanoma and 26 controls), 87 individuals for *MC1R* (38 melanoma and 49 controls), 77 individuals for *ASIP* (36 melanoma and 41 controls), 80 individuals for *NFE2L2* (35 melanoma and 45 controls), and 15 individuals for *SLC45A2* (6 melanoma and 9 controls). Expression of the ß-actin (*ACTB*) gene, which was detected in the skin of all individuals, was used for normalization, although we corroborated results by normalizing with a different gene (the glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*, gene). The fact that mRNA levels for some genes were not found in some cases indicates that some patients were not expressing some genes in normal skin or were expressing those genes in undetectable low levels. mRNA levels were highly correlated between most genes ($0.25 < r < 0.91$, $11 < n < 73$, $0.0001 < p < 0.091$), thus we tested the predictive capacity of melanoma incidence for each gene separately using generalized linear models.

Expression levels of the studied genes in the normal skin of controls and melanoma patients are shown in Fig. 1. The full model testing for the predictive capacity of *SLC7A11* mRNA levels, which included all phenotypic and behavioral factors potentially affecting melanoma risk and *NFE2L2* expression as predictors (Table 1), resulted in a significant effect of *SLC7A11* expression ($\chi^2_1 = 5.15$, $p = 0.023$). After removal of non-significant predictors, the final model explained a significant proportion of variability in melanoma incidence among individuals ($\chi^2_4 = 10.52$, $p = 0.032$), and again resulted in a significant effect of *SLC7A11* expression ($\chi^2_1 = 6.02$, $p = 0.014$). This effect indicated that expression levels were higher in the normal skin of melanoma patients than in controls (parameter estimate = -10.41; Fig. 1A). In addition to *SLC7A11* expression, a significant effect of Fitzpatrick skin type ($\chi^2_2 = 6.95$, $p = 0.031$) and a marginally non-significant effect of *NFE2L2* expression ($\chi^2_1 = 2.76$, $p = 0.096$), indicating a tendency of melanoma patients to show low expression levels (parameter estimate = 7.86), were other predictors remaining in the final model. A similar significant model ($\chi^2_4 = 11.03$, $p = 0.026$) was obtained when gene expression was normalized with *GAPDH* instead of *ACTB*, as it resulted in a significant effect of *SLC7A11* expression (parameter estimate = -13.19, $\chi^2_1 = 7.19$, $p = 0.007$), a marginally non-significant effect of Fitzpatrick skin type ($\chi^2_2 = 5.28$, $p = 0.071$) and a marginally significant effect of *NFE2L2* expression ($\chi^2_1 = 3.86$, $p = 0.049$). This indicates that results were independent of the housekeeping gene used for normalization.

Table 1
Phenotypic and behavioral characteristics of healthy individuals (control) and cutaneous melanoma patients included in the study. Mean ± sem values and ranges (in parentheses) are shown. For Fitzpatrick skin type and gender, the median (mode in parentheses) and the proportion of men, respectively, are shown instead.

	Control	Melanoma
Fitzpatrick skin type	III (III)	III (III)
Age (years)	62.16 ± 2.58 (18-92)	56.47 ± 2.54 (31-91)
Gender (% men)	56 % (28/50)	55 % (25/45)
Body mass (kg)	78.81 ± 1.92 (50-110)	76.05 ± 2.56 (50-115)
Frequency of sun exposure	1.48 ± 0.18 (0-6)	1.23 ± 0.14 (0-6)
History of sunburns	6.43 ± 2.54 (0-100)	7.00 ± 1.33 (0-30)
No. melanocytic nevi (0: < 50, 1: ≥ 50, 2: ≥ 100)	0.19 ± 0.08 (0-2)	0.93 ± 0.14 (0-2)

The same final model, *i.e.* controlling for the effect of Fitzpatrick skin type, did not result in a significant effect of expression levels when tested on *CTNS* (*ACTB*-normalization: $\chi^2_1 = 1.70$, $p = 0.192$; *GAPDH*-normalization: $\chi^2_1 = 1.68$, $p = 0.195$), *MC1R* (*ACTB*-normalization: $\chi^2_1 = 0.17$, $p = 0.680$; *GAPDH*-normalization: $\chi^2_1 = 0.08$, $p = 0.770$) or *ASIP* (*ACTB*-normalization: $\chi^2_1 = 0.97$, $p = 0.324$; *GAPDH*-normalization: $\chi^2_1 = 0.24$, $p = 0.625$). In *SLC45A2*, in contrast, the model resulted in a significant effect of expression level when normalized with *ACTB* ($\chi^2_1 = 4.71$, $p = 0.030$) similar to the effect found in *SLC7A11*, as *SLC45A2* expression was higher in the normal skin of melanoma patients than in controls (parameter estimate = -12.03; Fig. 1B). This result, however, should be taken with caution given the low sample size, as we only detected expression of *SLC45A2* in the skin of 15 individuals. Indeed, the effect of *SLC45A2* expression was not significant when normalized with *GAPDH* ($\chi^2_1 = 2.44$, $p = 0.118$).

In melanoma patients, *SLC7A11* expression was not related to the stage of melanoma (*ACTB*-normalization: $r = 0.002$, $n = 23$, $p = 0.993$; *GAPDH*-normalization: $r = 0.09$, $n = 24$, $p = 0.671$). *SLC7A11* expression was neither related to the thickness of melanoma lesions (*ACTB*-normalization: $r = 0.02$, $n = 23$, $p = 0.908$; *GAPDH*-normalization: $r = 0.19$, $n = 24$, $p = 0.369$).

3. Discussion

Our findings indicate that patients of cutaneous melanoma exhibit high *SLC7A11* expression in skin not affected by the tumor. According to our sample (Fig. 1A), ΔC_t values for *SLC7A11* below 6.5 cycles after normalization with *ACTB* or below 8.2 cycles after normalization with *GAPDH* should be considered as indicative of high risk of melanoma. No mutations in *SLC7A11* coding sequence are known to affect pigmentation in humans [32], suggesting that physiological effects promoted by alterations on the protein are more likely to be exerted by changes in its gene expression than by changes in its structure. Indeed, no single-nucleotide polymorphisms (SNP) in *SLC7A11* are related to melanoma risk [33]. Although a high *SLC7A11* expression has been reported as a causative factor leading to the incidence of different types of cancer [18–20],

this is, to our knowledge, the first time that it is found associated to melanoma. Notably, this is the first time that *SLC7A11* expression in non-tumorous tissues is related to the incidence of a cancer, opening a new alternative for the interpretation of the role of this gene in melanomagenesis. It must be considered, however, that only future prospective studies in which patients with different levels of *SLC7A11* expression are followed up will be able to establish the expression of this gene in epidermal melanocytes as a causative factor of skin melanoma.

High *SLC7A11* expression in normal skin may be the result of a high expression at the level of organism that is also present in melanoma cells, which may thus exhibit a high antioxidant capacity and resistance to ferroptosis derived from an impediment of p53 anti-tumor activity [17]. This may thus explain the association between high *SLC7A11* expression and melanoma incidence. However, the effect of *SLC7A11* expression was independent of the intrinsic antioxidant capacity of patients as given by the expression of the gene *NFE2L2*, meaning that, for a given level of antioxidant capacity, the incidence of melanoma increases with *SLC7A11* expression. Therefore, *SLC7A11* expression might have physiological consequences other than effects on the antioxidant capacity of cells, which makes likely an alternative explanation for the association between high *SLC7A11* expression and melanoma incidence. This explanation may be given by the fact that melanocytes use cysteine provided during *SLC7A11* activity not only for GSH synthesis but also for the synthesis of pheomelanin, which can potentially produce chronic oxidative stress [12,13]. This makes that, in melanocytes, a high *SLC7A11* expression can lead to increasing oxidative stress instead of decreasing it as in other types of cells [18–20]. This may explain why pigmentation phenotypes produced by large amounts of pheomelanin favor oxidative DNA damage and melanoma progression [10] and, similarly, the association that we found between high *SLC7A11* expression and melanoma incidence in normal skin. The lack of association that we found between *SLC7A11* expression and stage and thickness of melanoma lesions supports the view that the expression of this gene in normal skin increases the susceptibility to begin a tumorous process, but does not affect the progression of melanoma once the process is initiated.

The effect of *SLC7A11* expression was also independent of phenotypic and behavioral factors known to affect melanoma risk, especially the Fitzpatrick skin type, which increase with decreasing melanoma risk [27], and the frequency of sun exposure and sunburns [23]. Our results suggest that *SLC7A11* expression is a better predictor of melanoma incidence than these factors, which supports a direct biological relevance of this gene in melanomagenesis. This is further reinforced by the fact that the effect of *SLC7A11* explaining variability in melanoma incidence was significant while that of other genes involved in pheomelanin synthesis (*CTNS*, *MC1R* and *ASIP*) was not, despite a high correlation between the expression levels of all genes. Although other genes can influence the switch from the synthesis of pheomelanin to eumelanin in melanocytes, no other genes are known to be specifically involved in the synthesis of pheomelanin [34], thus our result points to a direct effect exerted by the activity of the antiporter xCT in melanocytes. The expression of *SLC45A2*, whose function is not clear but has been suggested to be involved in pheomelanin synthesis by transporting cysteine into melanosomes [31], also tended to be higher in melanoma patients than in controls, but this should be taken with caution as the low sample size prevented us from testing the relative importance of this gene's expression in relation to the other factors. Furthermore, no differences in *SLC45A2* expression between melanoma patients and controls were found when expression values were normalized with the gene *GAPDH* instead of *ACTB*. It is thus possible that the contribution of *SLC45A2* to melanoma risk is better explained by one SNP in this gene [33] than by its expression level.

These results may not only be used to determine melanoma risk. Most importantly, inducing downregulation of *SLC7A11* levels in individuals with a high expression of this gene should be considered as a new avenue to prevent melanoma progression. Recent findings in birds, which share the molecular pathway of pheomelanin synthesis with mammals, show that some species are able to downregulate *SLC7A11* in melanocytes when exposed to exogenous factors that generate oxidative stress and thus avoid oxidative damage [35]. Other species, in contrast, do not show lability in *SLC7A11* in response to environmentally induced oxidative stress and consequently exhibit a constrained antioxidant capacity [36]. This means that the downregulation of *SLC7A11* in melanocytes may actually be physiologically advantageous against oxidative stress induced by pheomelanin synthesis. Future studies should therefore propose alternatives to downregulate *SLC7A11* expresion in normal epidermal melanocytes.

4. Material and methods

4.1. Study design and skin sampling

This study was approved by the Research Ethics Committee of Virgen Macarena-Virgen del Rocío University Hospitals, Junta de Andalucía, on January 2017. Informed consent was obtained from all individuals included in the study after the nature and possible consequences of the study were explained, and the research was carried out according to the World Medical Association Declaration of Helsinki. All patients were attended at the Dermatology Department of the University Hospital Virgen del Rocio of Seville from January to December 2017. Epidermal samples were taken from normal remaining skin after surgical procedures during daily clinical practice. We included patients with cutaneous melanoma (cases) and patients with benign skin tumors (controls). In the case of melanoma patients, epidermal samples were obtained from skin regions near the melanoma lesion but not affected by the tumor (*i.e.*, healthy skin). Those patients affected with non-melanoma skin cancer were excluded to avoid confusing factors due to the similarities between basal cell carcinoma and melanoma etiology.

Fitzpatrick skin type (from I to IV) [27], age, sex, gender, frequency of sun exposure, history of sunburns and number of melanocytic nevi were registered for all patients (Table 1). The frequency of sun exposure was quantified as the total number of occasions in which patients are exposed to solar radiation during a year (considering exposure during leisure time, work, practicing sports, indoor tanning and phototherapy). The history of sunburns was considered as the total number of sunburns experienced by patients since childhood. Lastly, the number of melanocytic nevi was the total number of nevi considering all types observed in the patients, including congenital, compound, intradermal, dysplastic and junctional nevi. The number of melanocytic nevi was divided into three categories: 0 (< 50 nevi), 1 (≥ 50 nevi) and 2 (≥ 100 nevi) (Table 1).

We conducted histopathological analyses of the melanoma lesions of patients to determine the pathologic T stage of melanoma and the thickness of the lesions, the latter expressed as a measure of Breslow thickness (in mm). These analyses revealed that, out of 45 melanoma patients, 16 were in stage pT0 (melanoma *in situ*), 18 were in stage pT1a, two were in stage pT1b, three were in stage pT2a, one was in stage pT2b, two were in stage pT3a, one was in stage pT3b, and two were in stage pT4b. These analyses also revealed that most cases (30) corresponded to superficial spreading melanomas, while six corresponded to *in situ* melanomas, four to nodular melanomas, three to Spitzoid melanomas, one was a lentigo maligna melanoma, and one was an acral lentiginous melanoma.

A mean (± sem) amount of 36.4 ± 4.4 mg of epidermis was collected from each individual and immersed in RNAlater solution (Ambion, Thermo Fisher Scientific, Waltham, MA, USA). The samples were stored at -80 °C until analyses of gene expression.

4.2. Gene expression in skin

Although keratinocytes constitute the most abundant cellular component of the epidermis, its basal layer represents the main reservoir of melanocytes [14], thus the genetic material obtained from these samples corresponds to these cells to a large extent. Total RNA was

extracted using TRI Reagent (Ambion), and residual genomic DNA carry over was removed using the TURBO DNA-free kit (Ambion). The amount of extracted RNA was quantified with a Qubit 4 Fluorometer (Invitrogen, Thermo Fisher Scientific).

Complementary DNA (cDNA) was prepared from total RNA using RevertAid Reverse Transcriptase provided in the RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, Thermo Fisher Scientific). Quantitative real-time PCR (qPCR) was performed on cDNA for *SLC7A11*, *SLC45A2*, *CTNS*, *MC1R*, *ASIP* and *NFE2L2*. Reactions were performed using SYBR Green I Master in a LightCycler 480 System (Roche, Basel, Switzerland). Cycle threshold (C_t) levels were used as a measure of gene expression, with lower C_t values indicating higher mRNA levels and higher gene expression levels. C_t values for the housekeeping ß-actin (*ACTB*) gene were used for normalization by substraction to the C_t values for the other genes in the same samples (ΔC_t). Additionally, we normalized C_t values with other housekeeping gene (the glyceraldehyde-3-phosphate dehydrogenase gene, *GAPDH*) to corroborate the results obtained with *ACTB*-normalized C_t values.

Two replicates were obtained for each sample, and the mean value was used in the statistical analyses. Gene expression levels were unrelated to RNA concentration used in the analysis of the different genes (*SLC7A11*: $r = 0.12$, $n = 33$, $P = 0.487$; *SLC45A2*: $r = 0.18$, $n = 7$, $P = 0.691$; *CTNS*: $r = 0.02$, $n = 32$, $P = 0.894$; *MC1R*: $r = -0.21$, $n = 62$, $P = 0.100$; *ASIP*: $r = -0.09$, $n = 51$, $P = 0.514$; *NFE2L2*: $r = -0.15$, $n = 45$, $P = 0.311$; *ACTB*: $r = -0.22$, $n = 61$, $P = 0.090$; *GAPDH*: $r = 0.07$, $n = 62$, $P = 0.597$; RNA concentration could not be quantified in all samples). Sample sizes for gene expression levels differ between genes because we did not find detectable expression levels in some patients.

Gene primers were designed based on human refseq sequences (GenBank) using the Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The oligonucleotide primer sets used were as follows: 5′-CATCGTCCTTTCAAGGTGC-3′ and 5′-ATAGAGGGAAAGGG CAACC-3′ for *SLC7A11*; 5′-TTTCATGGGCCAGATTGTG-3′ and 5′- GAC TCCTCTTTCGTAGATGAG -3′ for *SLC45A2*; 5′- CGAAATGTGAGTCAA GCGTC-3′ and 5′- GGTTGCATTTAATGGTGGC-3′ for *CTNS*; 5′-CATCTC ACACTCATCGTCC-3′ and 5′-AGTTCTTGAAGATGCAGCC-3′ for *MC1R*; 5′-GAGATGACAGGAGCCTGAG-3′ and 5′-ATTTCTTGTTCAGCGCCAC-3′ for *ASIP*; 5′-ATGACAATGAGGTTTCTTCGG-3′ and 5′-CAATGAAGACT GGGCTCTC-3′ for *NFE2L2*; 5′-GAAGATCAAGATCATTGCTCCTC-3′ and 5′- ATCCACATCTGCTGGAAGG-3′ for *ACTB*; and 5′-TCAAGATCATCA GCAATGCC-3′ and 5′- CGATACCAAAGTTGTCATGGA-3′ for *GAPDH*.

4.3. Statistical analysis

Generalized linear models assuming a binomial response distribution and a logit link function were used to test the capacity of the expression level of each gene (ΔC_t; covariate) to explain variability in melanoma incidence (binomial response variable; 0-control, 1-melanoma) among individuals. Fitzpatrick skin type and gender were added as fixed factors to the models, and age, body mass, frequency of sun exposure, frequency of sunburns and number of melanocytic nevi were added as covariates. In the model for *SLC7A11*, ΔC_t for *NFE2L2* was also added as a covariate to control for the intrisic antioxidant capacity of the individuals. Starting with the full model, non-significant predictors were removed by a backward stepwise approach, establishing a *P*-value of 0.1 to abandon the model. Gene expression values were $\log_{10}$-transformed prior to analyses. As only one individual was assigned a Fitzpatrick skin type 1, it was considered a Fitzpatrick skin type 2 in the analyses. Pearson's correlation tests were used to investigate possible correlations between gene expression and the stage (considering increasing values from pT0 to pT4b) and thickness (in $\log_{10}$) of melanoma lesions.

Generalized linear models were checked for deviations from canonical assumptions using the over-dispersion coefficients. An over-dispersion value different from unity appears when the deviance of an observed response variable exceeds the nominal deviance, given the respective assumed distribution of errors and the proper link function. In our case, over-dispersion coefficients were close to unity (1.08 and 1.06 in the models for *SLC7A11* expression after normalization by *ACTB* and *GAPDH*, respectively), meaning that parameter estimates and related statistics are reliably computed. Analyses were made with STATISTICA 12.0 (StatSoft, Inc., Tulsa, OK, USA).

Authorship contributions

IG conceived the study and analyzed the data. IG and AI conducted gene expression analyses. MD, RCL, MTM and JBW examined patients and conducted skin sampling and histopathological analyses of melanoma lesions. IG wrote the manuscript.

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Declaration of Competing Interest

None.

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