Sun exposure and PDZK1 genotype modulate PDZK1 gene expression in normal skin

Human skin pigmentation results from the enzymatically controlled synthesis of melanin pigments in specialized organelles (melanosomes) produced within epidermal melanocytes, followed by their transfer to neighboring keratinocytes and their distribution throughout the epidermis. Constitutive skin pigmentation seems to be mostly genetically determined, being altered by numerous intrinsic and extrinsic factors affecting the epidermal melanin unit.

Ultraviolet (UV) radiation is the most significant factor influencing human skin pigmentation. The skin responds to UV radiation by stimulating melanin synthesis and thus increasing skin pigmentation over the basal constitutive level, with the aim of absorbing UV radiation and thus protecting the skin against sunlight's harmful effects. Basal cutaneous pigmentation can also be modulated by sex hormones, such as pregnancy-related hormones, through the regulation of melanin synthesis. In fact, hyperpigmentation of sun-exposed skin areas (melasma/chloasma) is frequently seen during pregnancy.

Epidemiological studies have shown that, although melasma development depends on the interaction of environmental (UV exposure) and hormonal (estrogens) factors, there is a clear genetic contribution, since melasma hyperpigmentation is more commonly seen in individuals with highly pigmented phenotypes (III/IV/V Fitzpatrick’s skin phototypes).

A recent study performed in skin tissue from melasma patients showed that estrogens increased levels of TYR expression, as well as the number of melanosomes transferred to keratinocytes, through upregulation of the PDZK1 gene. This gene encodes a 70-kDa scaffold protein with four PDZ-interacting domains that mediates numerous protein-protein interactions.

As the potential implication of PDZK1 in constitutive and/or facultative human skin pigmentation has not been studied yet, this work aimed to investigate whether PDZK1 expression in normal epidermis is influenced by different factors affecting melanogenesis. In this regard, a total of 96 cancer-free unrelated Spanish individuals donated a fresh-frozen normal skin sample. The study population is described in Table S1. Thirty-nine samples were obtained from chronically sun-exposed skin areas (neck, face, and hands), and 57 samples were collected from intermittently sun-exposed skin areas (back, chest, legs, and upper arms). Additional sampling details, data collection, and genetic analyses are provided in the Supplementary Methods online.

As PDZK1 has been shown to be upregulated by estrogens in melasma patients, levels of PDZK1 mRNA were compared between epidermal samples obtained from females with and without hyperpigmentation during pregnancy (Figure 1A). Twenty-five out of the 45 tissue-donating females had been pregnant at least once. No statistically significant differences in PDZK1 expression between females with pregnancy-related melasma and those who did not report skin darkening during pregnancy were found (P-value = .522). Probably, females showing melasma present an enhanced activation of PDZK1 in melasma regions of the face. The fact that in this study we lack skin samples from melasma-affected areas actually prevents us from performing this specific analysis. Changes in PDZK1 expression levels were not observed according to the presence or absence of sun-induced pigmented spots (P-value = .959; Figure 1B), as well as to the individual’s skin phototype (P-value = .089; Figure 1C). However, the levels of PDZK1 expression were significantly increased in individuals with dark basal skin color compared to fair-skinned individuals (P-value = .039; Figure 1D).

Then, levels of PDZK1 mRNA were compared between biopsies from chronically and intermittently sun-exposed skin areas. PDZK1 expression was significantly increased in chronically sun-exposed as opposed to intermittently sun-exposed skin samples (3.78-fold; P-value = 3.89 × 10⁻³; Figure 2A). Although this association analysis was not adjusted by individual variables (skin phototype or serum estrogen levels), this finding may provide an evidence for the role of cumulative sun exposure in the exacerbation of hyperpigmented conditions such as melasma. Since biopsies from normally pigmented skin taken by Kim et al (2012) were from areas not exposed to sunlight (retroauricular region), we suggest that it is the combination of both UV and estrogen effects that is needed for the upregulation of PDZK1 in epidermal cells.

Besides, the fact that there is a preferential appearance of melasma in darkly pigmented females demonstrates that pigmentation genes may play a significant role in the presence of melasma. For this reason, we set out to elucidate whether genetic variants in PDZK1 contributed to the variance of PDZK1 mRNA levels among individuals. SNP genotyping was performed as previously described. Out of eight PDZK1 SNPs analyzed, rs11576685 seems to be associated with PDZK1 expression variability (Table S2). In fact, individuals harboring one copy of the rare C allele in rs11576685 presented a significant increase in PDZK1 mRNA levels of 3.60-fold (P-value = .030), compared to individuals homozygous for the ancestral T allele. When focusing exclusively on chronically sun-exposed samples (skin areas where, as previously shown, levels of PDZK1 expression were constitutively high) an even higher increase in PDZK1 expression (3.91-fold; P-value = .024) was observed in rs11576685*C carriers.
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compared to homozygotes for the ancestral T allele (Figure 2B). However, these differences in PDZK1 expression levels according to the PDZK1 rs11576685 genotype were not observed in normal epidermal samples from intermittently sun-exposed skin areas (2.17-fold; P-value = .424; Figure 2B).

From these results, it can be gathered that the rs11576685 genotype seems to have a significant impact on PDZK1 expression, and therefore on melanin synthesis stimulation. Moreover, this PDZK1 upregulation seems to be enhanced in areas of the skin chronically sun-exposed such as the face, just where melasma typically arises, showing the essential role of UV exposure in the appearance of melasma.

This work also presents some limitations. Expression levels of genes usually show individual variation, and likely PDZK1 is no exception. Due to restrictions posed by the Ethics Committee, we were prevented from collecting more than one skin sample from each participant to compare PDZK1 levels from body sites differently exposed to UV radiation—a caveat partially mitigated by the relatively large number of skin samples collected compared to previous studies.\(^7\)
In conclusion, these results, together with the fact that PDZK1 has been shown to be upregulated in melasma via estrogens, suggest that genetic variants, UV exposure, and sex hormones exert a complex functional interaction to modulate PDZK1 expression in skin. This is actually strengthened by the fact that a higher prevalence of melasma has been observed in populations with darker pigmentation and higher skin phototypes, where there is greater exposure to UV radiation—including populations of Mediterranean origin.

ACKNOWLEDGEMENTS

We are indebted to all volunteers for giving their consent to participate in this study. We would like to thank Maria Torres for her expert technical support with the genotyping, carried out at the Spanish National Genotyping Centre in Santiago de Compostela (CEGEN-PRB2-ISCIII).

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

FUNDING INFORMATION

This work was supported by the Council of Education of the Generalitat Valenciana (Grant number GV/2016/156).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.