Title: Sun exposure and *PDZK1* genotype modulate *PDZK1* gene expression in normal skin

Short running title: *PDZK1* up-regulation by UV in normal skin

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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(1). Constitutive skin pigmentation seems to be mostly genetically determined(2), being altered by numerous intrinsic and extrinsic factors affecting the epidermal melanin unit(3).

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(4), with the aim of absorbing UV radiation and thus protecting the skin against sunlight's harmful effects(5).

Basal cutaneous pigmentation can also be modulated by sex hormones, such as pregnancy-related hormones, through the regulation of melanin synthesis(3). In fact, hyperpigmentation of sun-exposed skin areas (melasma/chloasma) is frequently seen during pregnancy(6). 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)(6).

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(7). This gene encodes a 70-kDa scaffold protein with four PDZ-interacting domains that mediates in numerous protein-protein interactions(8).

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 95 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 56 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(7), levels of *PDZK1* mRNA were compared between epidermal samples obtained from females with and without hyperpigmentation during pregnancy (Fig 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=0.522). 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=0.959; Fig 1b), as well as to the individual's skin phototype (*P*-value=0.089; Fig 1c). However, the levels of *PDZK1* expression were significantly increased in individuals with dark basal skin color compared to fair-skinned individuals (*P*-value=0.039; Fig 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}; Fig 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(6). 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(6). 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(9). 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=0.030), 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=0.024) was observed in rs11576685*C carriers, compared to homozygotes for the ancestral T allele (Fig 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=0.424; Fig 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(7), 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 have been observed in populations with darker pigmentation and higher skin phototypes, where there is greater exposure to UV radiation – including populations of Mediterranean origin(6,10).

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Figure Legends

Fig 1. Real-time PCR of *PDZK1* mRNA expression in human normal epidermal samples. Box plots comparing *PDZK1* expression levels between (a) females with or without pregnancy-related hyperpigmentation, (b) individuals with or without sun-induced spots, (c) individuals with III-IV-V or I-II Fitzpatrick's skin phototype, and (d) individuals with dark or fair/pale basal skin color. Dots represent the *PDZK1* mRNA levels relative to β-actin ($2^{-\Delta Ct}$) of each epidermal sample analyzed. Black dots represent intermittently sun-exposed epidermal samples, while grey dots represent samples from chronically sun-exposed areas. Differences in mRNA *PDZK1* levels between different subsets were assessed using one-way ANOVA adjusted by the UV exposure of the skin area, age and/or sex.

Fig 2. Differences in *PDZK1* expression levels in human normal epidermal samples. Bar plots represent (a) fold-change of the relative *PDZK1* mRNA expression in chronically sun-exposed epidermal samples compared to samples from intermittently sun-exposed skin areas, and (b) fold-change of the relative *PDZK1* mRNA expression in both chronically and intermittently sun-exposed epidermal samples from carriers of the derived C allele compared to individuals homozygous for the ancestral T allele. Data represents mean ± SEM for all individuals included in each population subgroup. The delta-delta Ct method was used to calculate the relative changes in *PDZK1* expression levels between different population subsets. Differences in mRNA *PDZK1* levels between different subsets were assessed using one-way ANOVA adjusted by sex and age.