Sex and *MC1R* variants in human pigmentation: differences in tanning ability and sensitivity to sunlight between sexes

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Abbreviations:

UV, ultraviolet; SNP, single nucleotide polymorphism; RHC, red hair colour associated variants; *P*, *P*-value; OR, odds ratio; CI, Confidence Interval.

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Human skin acts as a biological active barrier to the external environment, including exposure to UV radiation – clearly the major risk factor for melanoma. Sex differences are well-known regarding melanoma, with females presenting lower incidences, less metastases and better survival rates than males [1].

Both genetics and sex hormones contribute to sexual differences in skin aging, pigmentation, UV-light sensitivity, and melanoma incidence and outcome [2]. Oestrogens accelerate wound healing, improve inflammatory disorders, increase skin thickness, protect from skin photoaging, and induce the activation and expression of genes involved in melanin synthesis [3].

Basal skin pigmentation, via melanin synthesis, darkens in response to sunlight, thus fulfilling its protective role against further irradiation-induced damage [2]. Therefore, sex disparity in melanoma epidemiology might be explained by sex differences in tanning ability and skin sensitivity to UV-light exposure.

With the purpose of shedding some light on these questions, we evaluated a total of 1,112 individuals (515 males and 597 females) of Spanish origin for pigmentary traits related to tanning ability and sun sensitivity – skin phototype, history of sunburns, presence of solar lentigo and number of naevi. A brief summary of the materials and methods used in this work is available in the Supplementary Material.

When these phenotypic traits were analysed according to sex, the percentages of skin phototypes and naevus numbers appeared to be significantly different between the two sexes (Table S1). The percentage of phototypes I-II was notably higher in females than in males (48.06% vs. 38.17%, P=8.35x10-3). Regarding naevus number, the percentage of naevi was

remarkably lower in females than in males (68.34% vs. 59.03%, P=1.25x10-3). No significant differences between the sexes were observed for history of sunburns and presence of lentigos. A meta-analysis was performed to compare our results to previously published data (Figure 1). The guidelines followed to perform the meta-analysis are briefly explained in the Supplementary Material. We searched for studies conducted in Caucasian populations presenting phenotypic data stratified by sex (Supplementary References S1-7). When all individuals included in these studies were analysed together, the difference between females and males was extremely significant for both skin phototype (OR=0.75, 95% CI: 0.68-0.83, P=1.90x10-9) and naevi (OR=1.42, 95% CI: 1.30-1.55, P=1.11x10-15). The results obtained in this Spanish study were concordant with the results of the meta-analysis.

Our results are consistent with earlier anthropological studies indicating that females have less tanning ability, and therefore lower phototypes, than males in most populations, as males show greater pigmentation contrast between exposed and unexposed skin regions [4,5]. Furthermore, Jacobs and cols. (2015) showed that females presented a much higher prevalence of facial sun spots than males, suggesting that females are more severely affected by sun exposure than males independently of genotype [6]. These differences could be the result of socio-cultural reasons, as males tend to spend more time outdoors; physiological reasons, as males have thicker skin and increased number of blood vessels; differential tanning, as no sex difference in basal skin pigmentation has been shown; and hormonal factors, as oestrogens stimulate pigmentation while androgens have an inhibitory effect on melanocytes [4,5].

An elevated naevi number, a major predictor factor for melanoma occurrence, is directly correlated with high levels of sun exposure [7]. As expected, females present fewer naevi than

males. These results might be in apparent conflict with those obtained for skin phototype, since naevus prevalence has been significantly associated with the propensity to burn slightly and tan lightly [8]. However, tanning degree – the difference between unexposed and exposed skin colour – has also been positively associated with naevus count [7]. This inconsistency might arise as a consequence of the individual perception of overall darkness of tan when self-reported questionnaires are used to collect information on pigmentation characteristics. As a result, this naevus count sex-specific difference might be attributable to higher acquired sun exposure levels in males than females, and not to genetic effects.

Previous studies have evidenced sex-differentiated genetic effects for anthropometric traits, serum metabolite concentrations, human pain inhibition, human pigmentation, and melanoma risk (Supplementary References S8-S14).

Considering the importance of genetics in UV-light response, we focused on identifying a possible genetic cause explaining phenotypic differences between sexes. We genotyped five SNPs involved in human pigmentation pathways: rs12913832 (located in the HERC2/OCA2 region), rs1800407 (OCA2 gene), rs16891982 (SLC45A2 gene), rs1393350 (TYR gene), and rs12203592 (IRF4 gene). The coding region of MC1R gene was also studied by direct sequencing, classifying MC1R functional variants as RHC (red hair colour) and non-RHC associated variants.

Genotype association analyses were performed via logistic regression for each SNP as well as for sex. To assess for possible confounding effects, regression estimates were adjusted by executing a multivariate logistic regression. After adjustment, skin phototypes I-II were significantly associated with MC1R RHC variants at Bonferroni-corrected level ($P=2.96\times10^{-4}$), but were also moderately associated with female sex ($P=2.11\times10^{-2}$). No association was

observed between naevi number and any of the genetic variants studied. However, being male remained significantly associated with having ≥25 naevi (P=1.12x10-2), meaning that male sex might be a factor contributing to high naevus count (Table 1A).

The protein encoded by the MC1R gene functions as a receptor for α-MSH, a hormone produced in the pituitary gland that depends on oestrogen levels. Interestingly, MC1R RHC variants presented differences in genetic effects by sex, with greater effects in skin phototype in females than in males (Table 1B). That is, females carrying an RHC variant tended to exhibit significant lower phototypes than males with the same MC1R genotypes (OR=2.20, P=0.029). In a previous study, MC1R genotype revealed a significant greater influence on analgesia from pentazocine in females than in males [9]. Furthermore, mutations in another gene of the melanocortin receptor family, MC4R, presented about twice as stronger effect on body mass index in females than in males [10].

In summary, this study supports previous evidence that sex might be a factor explaining variations in tanning ability and sensitivity to sunlight between females and males in Caucasian populations. Additionally, we suggest that MC1R genetic effects might contribute to these sexspecific differences in skin phototype.

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Table 1

A) Genotypic association with phenotypic traits

			Skin Phototype I/II				Naevus number ≥25			
			Non-adjusted		Adjusted[a]		Non-adjusted		Adjusted[a]	
Gene	**SNP ID**	**Allele/Factor**	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**
HERC2	rs12913832	C	0.89	1.02 (0.81-1.27)	0.57	1.07 (0.84-1.37)	0.44	1.07 (0.89-1.28)	0.76	1.03 (0.85-1.25)
OCA2	rs1800407	T	0.24	1.23 (0.87-1.73)	0.59	1.11 (0.76-1.63)	0.47	0.90 (0.68-1.19)	0.54	0.91 (0.67-1.24)
TYR	rs1393350	T	**3.32E-02**	**1.32 (1.02-1.69)**	0.06	1.29 (0.98-1.68)	0.34	0.90 (0.73-1.12)	0.31	0.89 (0.72-1.11)
SLC45A2	rs16891982	C	0.16	1.31 (0.90-1.90)	0.35	1.21 (0.81-1.78)	0.41	1.13 (0.85-1.50)	0.33	1.15 (0.89-1.54)
IRF4	rs12203592	A	0.82	0.97 (0.72-1.30)	0.75	1.05 (0.76-1.46)	0.72	1.05 (0.81-1.35)	0.89	0.98 (0.75-1.28)
MC1R	RHC variants	RHC	**3.00E-06***	**2.00 (1.49-2.69)**	**2.96E-04***	**1.89 (1.34-2.69)**	0.61	1.07 (0.83-1.38)	0.34	1.14 (0.87-1.48)
	Sex	Male	**8.57E-03**	**0.67 (0.49-0.90)**	**2.11E-02**	**0.65 (0.45-0.94)**	**1.28E-03***	**1.37 (1.05-1.78)**	**1.21E-02**	**1.45 (1.08-1.93)**
	Skin Colour	Fair/Pale	**6.11E-07***	**2.20 (1.61-3.00)**	**1.36E-04***	**2.03 (1.41-2.96)**	**5.98E-03**	**1.42 (1.11-1.83)**	**2.33E-02**	**1.40 (1.05-1.88)**
	Sunburn History	Yes	**5.12E-07***	**2.32 (1.67-3.22)**	**5.10E-05***	**2.20 (1.50-3.24)**	**4.32E-13***	**2.67 (2.05-3.48)**	**1.46E-12***	**2.94 (2.18-3.97)**

B) Sex differences in skin phototype within *MC1R* genotype

	***MC1R* Wild-type**				***MC1R* RHC variants**			
	Skin phototype[b]				**Skin phototype[b]**			
Sex	**III-IV**	**I-II**	**OR**	***P*-value[c]**	**III-IV**	**I-II**	**OR**	***P*-value[c]**
Male	65.70 %	34.30 %	reference		51.20 %	48.80 %	reference	
Female	58.00 %	42.00 %	1.39 (0.97-1.98)	0.071	34.80 %	65.20 %	**2.20 (1.13-4.29)**	**0.029**

Abbreviations: SNP, single nucleotide polymorphisms; OR, Odds Ratio; CI, Confidence Interval; RHC, red hair colour

RHC variants include both homozygotes and heterozygotes

Bold indicates statistically significant results

* *P*-value significant at Bonferroni-corrected threshold of 0.05/9 = 0.0055

[a] Multivariate logistic regression analysis. Results adjusted by including all the potential risk factors in the model, considering as risk factors all six SNPs, sex, skin colour and history of sunburns

[b] Percentages of the all individuals in each subgroup

[c] *P*-values for Fisher's exact test, estimating sex differences in skin phototype within each *MC1R* genotype

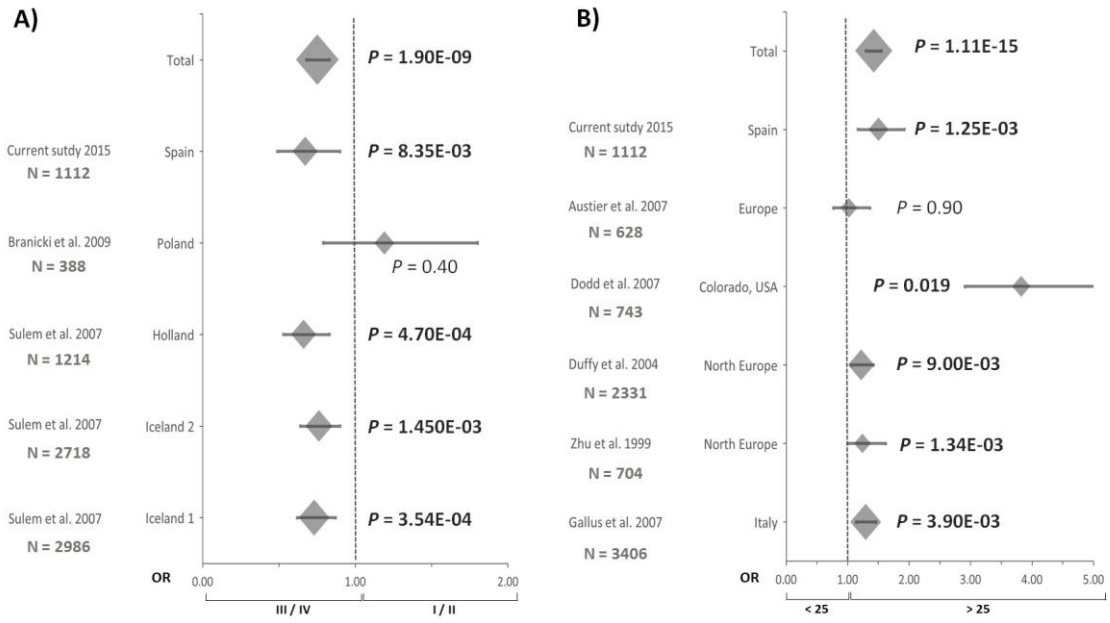

Figure 1. Sex-specific meta-analysis of (a) skin phototype and (b) naevus number in different Caucasian populations. Since femaleness was set as the reference, the results show male ORs. Diamond shapes represent odds ratio in each study and in the pooled analysis (Total). Diamond size is proportional to the number of individuals, and error bars represent 95% confidence intervals. Bold on *P*-values denotes statistically significant results. N refers to the total individuals analysed in each study. Total: results attained by taking into account all populations.