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Distribution of *MC1R* variants among melanoma subtypes: p.R163Q is associated with Lentigo Maligna Melanoma in a Mediterranean population

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Abstract

Background—Cutaneous melanoma tumour is classified into clinico-histopathological subtypes which may be associated with different genetic and host factors. Variation in the MC1R gene is one of the main factors of risk variation in sporadic melanoma. The relationship between MC1R variants and the risk of developing a specific subtype of melanoma has not been previously explored.

Objective—to analyze whether certain *MC1R* variants are associated with particular melanoma subtypes with specific clinico-histopathological features.

Methods—An association study between MC1R gene variants and clinico-pathological subtypes of primary melanoma derived from 1679 patients was performed.

Results—We detected 53 *MC1R* variants (11 synonymous and 43 non-synonymous). Recurrent non-synonymous variants were p.V60L (29.9%), p.V92M (11.7%), p.D294H (9.4%), p.R151C (8.8%), p.R160W (6.2%), p.R163Q (4.2%) p.R142H (3.3%), p.I155T (3.8%), p.V122M (1.5%) and p.D84E (1%). Melanoma subtypes showed differences in total number of *MC1R* variants (P-value=0.028) and number of Red hair colour variants (P-value=0.035). Furthermore, an association between the p.R163Q variant and lentigo maligna melanoma subtype was detected under a dominant model of heritance (OR: 2.16 95% CI: 1.07–4.37; P-value=0.044). No association was found between p.R163Q and skin Fitzpatrick's phototype, eye colour or skin

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colour indicating that the association was independent of the role of MC1R in pigmentation. No association was observed between *MC1R* polymorphisms and other melanoma subtypes.

Conclusion—Our findings suggest that certain *MC1R* variants could increase melanoma risk due to their impact on pathways other than pigmentation and therefore be linked to specific melanoma subtypes.

INTRODUCTION

Cutaneous melanoma (MM) has been classically classified into distinct subtypes based on histological appearance, biological behaviour and epidemiological features[1, 2]. Later on, this classification lost relevance because there was often a significant overlap between types and as it lacked prognostic value. Nevertheless, some of these variants show characteristic clinical features and may be associated with different risk factors. Differences according to anatomical location of primary tumour, UV pattern of exposure and somatic genetic alterations have also been identified[3,4]. Overall, superficial spreading melanomas (SSM) develop mostly on trunk and extremities associated with acute-intermittent sun-exposure patterns. In contrast, lentigo malignant melanomas (LMM) usually originate on the face or chronically exposed areas. The incidence of both subtypes of melanoma increase continuously over time in populations from European origin[5]. Acral lentiginous melanomas (ALM), located on palms, soles, and subungual sites are not associated with sun exposure, its incidence being similar in dark and fair skin populations. The epidemiology of nodular melanomas (NM) is not clearly associated with sun exposure maintaining a stable incidence and mortality[6]. These associations may explain the epidemiological differences detected in different populations/studies. Whilst in most studies intermittent/recreational sun exposure and sunburns are consistently associated with melanoma risk (probably associated with SSM), in a few studies melanoma is also associated with occupational sun exposure, cumulative lifetime sun exposure or markers of such an exposure[7,8]. This risk seems to be mostly associated with LMM as, in areas with high levels of sun exposure, LMM becomes the more frequent subtype of melanoma[5].

Risk factors for melanoma development also include genetic and host characteristics such as fair skin, family history of melanoma, eye and hair pigmentation[9–11].

The pigmentation-related melanocortin receptor 1 (*MC1R*), which is the major contributor to pigmentation diversity in humans, is also a risk factor for melanoma[12]. The gene is highly polymorphic, with more than 100 variants, many being non synonymous[13]. A meta-analysis identified five *MC1R* variants (p.D84E, p.R142H, p.R151C, p.R160W, p.D294H) associated with the red hair colour phenotype (R) which is characterized by fair pigmentation (fair skin, red hair and freckles), and by sun sensitivity (poor tanning response and solar lentigines)[14]. Functional studies have revealed the complexity of *MC1R* genomic variation. Allelic variants show differences in loss of function among R and non-red hair colour variants (r)[13]. Furthermore, distribution of the allelic frequency of recurrent variants differs significantly across populations. Such differences are not only exclusively detected when comparing dark versus fair-pigmented populations. Allelic frequencies of p.V60L and p.D294H variants are different even when comparing different dark-pigmented populations[15].

Few studies have focused on the role of *MC1R* variants in melanoma beyond the study of melanoma risk in individuals. An association between germline *MC1R* status and presence of somatic BRAF mutation in melanoma was found[16, 17]. However, these findings have not been confirmed by other studies[18–20], illustrating the complexity of cross-talk between *MC1R* variants, UV exposure pattern, melanoma subtype and somatic alterations which can be over-represented in certain combinations.

The aim of this study was to analyze whether some *MC1R* variants are associated to particular clinico-histopathological melanoma subtype.

MATERIAL AND METHODS

Samples

An observational retrospective study including a series of 1679 melanoma patients from two hospital-based series was designed. Inclusion criteria were patients with confirmed histopathological information of the tumour. In the patients with multiple primary melanomas (MPM), only patients with histopathological subtype information for all tumours and information available on the time of occurrence for each were included. All patients were treated and controlled at the Melanoma Unit in the Hospital Clinic of Barcelona (Barcelona, Spain) and in the Instituto Valenciano de Oncologia (Valencia, Spain).

The study of *MC1R* variants was approved by the institutional review board of both Hospitals and informed consent from all study participants was obtained.

The outcome variable of the study was the histopathological melanoma subtype. For the purpose of the study only the following subtypes were considered: lentigo maligna melanoma (LMM), superficial spreading melanoma (SSM), nodular melanoma (NM) and acral lentiginous melanoma (ALM). Patients with other unknown or unclassified tumours were excluded from the study.

In the analysis of *MC1R* variants and histopathological subtypes, the MPM patients were included only once in each analysis: a) in the analysis of the total number and type of variants, patients were included based on the histopathological subtype of the first developed melanoma; b) in the analyses of each of the 10 more frequent variants and melanoma subtype (LMM, SSM or NM), MPM patients were re-classified according to whether they had had, or not, the specific melanoma subtype at any time. Such a strategy avoids the inclusion of the same patient more than once in the analysis (consequently we did not increase the frequency of *MC1R* variants).

As potential confounders, the following variables were considered: sex information, age of onset, hair colour (red, blonde or brown/black), skin phototype according to the classification by Fitzpatrick (I–II vs. III–V)[21] and eye colour (dark/brown vs. green/blue).

MC1R molecular screening

Samples from Melanoma unit-Hospital Clinic of Barcelona were amplified using primers described by Chaudru et al.[22]. PCRs conditions were: initial denaturizing step at 95°C for 5 min, followed by 35 cycles (95°C for 1 min, 55°C for 1 min, 72°C for 1 min), and a final extension at 72°C for 10 min and maintaining at 4°C. Specific internal *MC1R* primers were designed to analyze the entire coding sequence (INT-F: TACATCTCCATCTTCTACGC and INT-R: GTGCTGAAGACGACACTG). Samples from Instituto Valenciano de Oncologia-Valencia were genotyped as described in Scherer D et al.[23].

Statistical analysis

MC1R variants were classified as red hair colour (R) or non-red hair colour (r) according to previously reported criteria[14]. Therefore, *MC1R* variants classified as R were p.D84E, p.R142H, p.R151C, p.R160W and p.D294H. All other non-synonymous *MC1R* variants were classified as r. Synonymous variants were considered as wild-type *MC1R* alleles. For the purpose of this study, only variants with an observed frequency of at least 1% were analysed. *In-silico* analysis of each variant to predict the effect of the amino acid change in

both protein structure and *MC1R* function was carried out using PolyPhen-2 version 2.2.2[24]. Correlation between the number of *MC1R* variants and confounding variables was calculated by cross-tabulations and Pearson's χ^2 using IBM SPSS Statistics 20. Genetic data was analyzed using SNPStats software[25]. Multiple logistic regression models [Codominant, Dominant, Recessive, Overdominant and log-additive] were performed for odds ratio (OR), 95% confidence interval (CI), and P-value. Both Akaike information (AIC) and Bayesian Information Criterion (BIC) were used to choose the model of inheritance that best fit the data. In some analyses, the model was adjusted by confounding variables that are associated with the MC1R variant of interest. P-values less than 0.05 were considered as statistically significant. All tests were two-sided and Bonferroni correction for multiple comparisons was applied in all P-values.

RESULTS

MC1R genotyping was carried out in 1679 patients that met the selection criteria, 1428 (85%) were single primary (SPM) cases and 251 (15%) were MPM cases (mean number of tumours=2.34). The SPM subgroup included 979 SSM patients (68.6%), 249 NM patients (17.4%), 118 LMM patients (8.3%) and 82 ALM patients (5.7%).

The MPM subset included 198 (78.9%) patients who developed 2 MMs, 36 (14.3%) patients who developed 3 MMs and 8 (3.2%) and 9 (3.6%) patients who developed 4 and ≥5 MMs, respectively. MPM patients displayed a total of 588 MMs, 83.5% of them were SSM (491/588), 8.2% of them were LMM (48/588), 6.8% of them were NM (40/588,) and 1.5% of them ALM (9/588,). The frequencies for each subtype in the subset of first MMs in MPM patients (N=251; LMM 6.8%, SSM 82.9%, NM 8.3% and ALM 2%) were statistically not different to those observed in the subset of subsequent MMs diagnosed in MPM patients (N=337; LMM 9.2%, SSM 84%, NM 5.6% and ALM 1.4%).

The rate of the histopathology subtype concordance was evaluated in MPM patients. The rate of concordance was 85.5% in SSM patients (178/208), 35.3% in LMM patients (6/17) and 4.8% in NM patients (1/21). No patients with a firstly ALM (5) develop other ALMs.

The study identified 53 *MC1R* variants (11 synonymous and 43 non-synonymous) most being detected in a small number of patients or restricted to one (Table 1). Thirteen *MC1R* variants had not been identified in previous studies. Among synonymous variants, the highest frequency was observed for p.T314T (17.6% of patients). Recurrent non-synonymous variants in the set of 1679 melanoma patients with a frequency of at least a frequency of 1% were p.V60L (29.9%), p.V92M (11.7%), p.D294H (9.4%), p.R151C (8.8%), p.R160W (6.2%), p.R163Q (4.2%) p.R142H (3.3%), p.I155T (3.8%), p.V122M (1.5%) and p.D84E (1%). Analyses of *MC1R* variants and phenotypical features and histopathological subtype of tumour were carried out only in those 10 variants.

Overall, skin phototype information was available in 94.8% of cases, eyes and hair colour in 90.5% and 93.4%, respectively. The p.R142H, p.R151C, p.R160W and p.D294H variants were statistically significant associated to red hair colour and fair skin (phototype I or II) under a co-dominant model of heritance (Table 2). The association of *MC1R* variants and eye colour was restricted to p.R142H which was associated to fair eye colour (green or blue) under a dominant model of heritance (OR= 2.07: 95%CI=1.18-3-65; p=0.011).

Differences in terms of number and type of *MC1R* variant (r or R) were analyzed. Skin phototype and hair colour showed differences in the total number of variants (P-value<0.001). When the analysis was focused on the number of r variants, there was no statistically significant association with phenotypic characteristics. However, a trend was

observed between fair skin and presence of ≥2 variants (p =0.041). In contrast, number of R variants was associated to both hair colour and skin phototype (P-value<0.001). Overall, 89.2% of red hair patients carried at least one R variant and 54.1% of them carried ≥2 R variants.

According to the number of *MC1R* variants and histopathological melanoma subtype (Table 3) differences were observed in total number of *MC1R* variants (P-value =0.028), mainly due to the low frequency of *MC1R* variants in patients with ALM subtype. Also, differences in number of R variants was observed (P-value=0.035), showing a lower number of variants in both ALM and NM subtypes.

Association of histopathological subtype and specific recurrent *MC1R* variant was restricted to those subtypes associated with a sun exposure pattern (SSM, LMM and NM). Logistic regression model was adjusted by number of primary tumours, sex, age of onset and phenotypical characteristics. No statistical significant association was found between certain *MC1R* variants and SSM or NM. In contrast, an association was detected between the p.R163Q variant and LMM development under a dominant model of heritance (OR: 2.16 95%CI: 1.07–4.37; P-value=0.044) (Table 4).

DISCUSSION

Since Clark et al. classified melanomas into three distinct subtypes[2] and thereafter, a fourth subgroup was proposed[26], several studies have elucidated epidemiological and clinical features which are more associated with a particular histopathological subtype[27–30]. Some of these differences can be attributed to variation of UV exposure (chronic sun exposition or intermittent)[4].

To date, polymorphisms in the *MC1R* gene are major determinants of hair and skin colour[31]. Furthermore, *MC1R* polymorphisms play a role in sun sensitivity and low tanning ability in response to UV radiation independently of skin colour[32]. Thus, certain *MC1R* variants could be related to particular histopathological group of melanomas associated to different patterns of UV radiation.

In the present study, the genomic status of *MC1R* gene from 1679 melanoma patients was analyzed according to their histopathological melanoma subtypes. In the study, patients with multiple primary melanomas were also included as different melanomas from the same patient may be considered as independent occurrences of the disease[33]. In the association studies for number of variants just the histopathological classification of the first melanoma was considered. When evaluating the association of each subtype with each prevalent *MC1R* variant, MPM patients were categorized according to whether they had had, or not, at least one tumour of the specified subtypes at any time (i.e., if a patient had developed two LMMs, in the LMM analysis the patient was recorded once). The systematic exclusion of 15% of patients with MPM in genetic studies could occult important data concerning differences in genetic background, more present in MPM patients compared to SPM, such as the occurrence of dysplastic nevi or UV exposure[34, 35].

Analysis of *MC1R* variants and phenotypical characteristics was carried out to find previous well-established associations[14] and to consider them in the posterior analysis. Variants p.R142H, p.R151C, R160W and p.D294H variants associated with red hair and fair skin. The p.R142H variant was also associated with patients with green/blue eyes. Previous studies have found no effect of *MC1R* genotype on eye colour. However, an epistatic interaction between *MC1R* and the *OCA2* gene, which is a significant determinant of eye

colour, has been postulated[36, 37]. Our study adds further evidence to the modifier effect of *MC1R* alleles on eye colour.

In the present study, the total number of *MC1R* variants and number of RHC variants were higher in both LMM and SMM subtypes. An increased prevalence of *MC1R* variants in tumours on intermittently exposed sites has been frequently observed. Unfortunately, the *MC1R* distribution according to anatomic site was not addressed in our study.

The most relevant finding was the association between p.R163Q and LMM; independent of phenotype features (it was not associated with Fitzpatrick skin photoype, eye or hair colour) which suggests that certain variants could be linked to specific melanoma subtypes. Different functional influence among *MC1R* variants has been shown in terms of cell surface expression, functional ability or dominant negative activity of pigment related pathway[13, 38–40].

In addition to adenylate cyclase signalling, stimulation of *MC1R* also activates the mitogen-activated protein kinase (MAPK) pathway[41] and regulates target genes involved in inflammation through the NF-Kb pathway[42]. Thus, interpretation of the effect of *MC1R* alleles in melanoma beyond its role in pigmentation, such as the relation between p.R163Q and LMM subtype, is complex. Furthermore, differences in frequency and type of variants between populations could result in variations in genotype-phenotype correlation[23]. The frequency of the p.R163Q variant is highly variable among populations, being higher in Asian compared to European origin populations, but also there are differences among European populations[15]. In the Japanese population, p.R163Q and p.V92M have been related to skin lesions associated with UV damage such as freckles and solar lentigines[43]. Previous studies have suggested a relation between these variants and chronic UV radiation in populations of European origin. The p.V92M variant has been associated with severe photoaging of facial skin, independent of the presence of other minor and major variants, in European women[44]. Furthermore, variant p.R163Q has been related to non-melanoma skin cancer development in Europeans, underlying its possible role in tumours related to chronic sun exposure[45]. Thus, our finding that p.R163Q is related to LMM susceptibility in our population may be a consequence of the role of this variant in skin photodamage and photoaging since a propensity to solar lentigines is a strong predictor of LMM and it is not associated to another subtype of melanoma[46]. Interestingly, both p.R163Q and V92M presented a benign score in the in-silico analysis (Table 1) and are considered “pseudo-alleles” with no significant effect on eumelanin synthesis[47]. Thus, the biological relevance of these variants could be related with a non canonical *MC1R* pathway. Although, variant p.R163Q does not display changes in either surface expression or cAMP signalling, a selective decrease in MAPK activation has been recently described[48]. Thus, the cross-talk between specific *MC1R* variants and MAPK pathway activation could be responsible in part for the differing results reported for correlation between *MC1R* and mutant *BRAF* in melanoma[16–20]. Furthermore, the majority of studies have been conducted using a pigment related classification of *MC1R* variants and mostly without histopathological information of the tumour subtype. As a previous study suggested[49], our data supports that these differences could be due to unique effects of specific *MC1R* variants, the frequencies of which differ somewhat among populations.

In conclusion, the *MC1R* variant p.R163Q showed differences among histopathological melanoma subtypes, showing a positive association with LMM. Moreover, these findings suggest that differences exist beyond the role of *MC1R* variants in the pigment synthesis process. Thus, common variants could be responsible in part for the risk of LMM in non-fair skin population. Further studies should be directed to elucidate the mechanisms by which

MC1R variants play a role in susceptibility to melanoma independent to its relationship with phenotypic traits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Marks R. Epidemiology of melanoma. Clin Exp Dermatol. 2000; 25:459–63. [PubMed: 11044179]
2. Clark WH Jr, From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res. 1969; 29:705–27. [PubMed: 5773814]
3. Elwood JM, Hislop TG. Solar radiation in the etiology of cutaneous malignant melanoma in Caucasians. Natl Cancer Inst Monogr. 1982; 62:167–71. [PubMed: 7167183]
4. Holman CD, Armstrong BK. Cutaneous malignant melanoma and indicators of total accumulated exposure to the sun: an analysis separating histogenetic types. J Natl Cancer Inst. 1984; 73:75–82. [PubMed: 6588237]
5. Forman SB, Ferringer TC, Peckham SJ, Dalton SR, Sasaki GT, Libow LF, Elston DM. Is superficial spreading melanoma still the most common form of malignant melanoma? J Am Acad Dermatol. 2008; 58:1013–20. [PubMed: 18485983]
6. Shaikh WR, Xiong M, Weinstock MA. The contribution of nodular subtype to melanoma mortality in the United States, 1978 to 2007. Arch Dermatol. 2011; 148:30–6. [PubMed: 21931016]
7. Rodenas JM, Delgado-Rodriguez M, Herranz MT, Tercedor J, Serrano S. Sun exposure, pigmentary traits, and risk of cutaneous malignant melanoma: a case-control study in a Mediterranean population. Cancer Causes Control. 1996; 7:275–83. [PubMed: 8740740]
8. Ballester I, Oliver V, Banuls J, Moragon M, Valcuende F, Botella-Estrada R, Nagore E. Multicenter Case-Control Study of Risk Factors for Cutaneous Melanoma in Valencia, Spain. Actas Dermosifiliogr. 2012
9. Tucker MA, Goldstein AM. Melanoma etiology: where are we? Oncogene. 2003; 22:3042–52. [PubMed: 12789279]
10. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, Melchi CF. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer. 2005; 41:45–60. [PubMed: 15617990]
11. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Zanetti R, Masini C, Boyle P, Melchi CF. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer. 2005; 41:2040–59. [PubMed: 16125929]
12. Rees JL. Genetics of hair and skin color. Annu Rev Genet. 2003; 37:67–90. [PubMed: 14616056]
13. Garcia-Borron JC, Sanchez-Laorden BL, Jimenez-Cervantes C. Melanocortin-1 receptor structure and functional regulation. Pigment Cell Res. 2005; 18:393–410. [PubMed: 16280005]

14. Raimondi S, Sera F, Gandini S, Iodice S, Caini S, Maisonneuve P, Fargnoli MC. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. Int J Cancer. 2008; 122:2753–60. [PubMed: 18366057]
15. Gerstenblith MR, Goldstein AM, Fargnoli MC, Peris K, Landi MT. Comprehensive evaluation of allele frequency differences of MC1R variants across populations. Hum Mutat. 2007; 28:495–505. [PubMed: 17279550]
16. Landi MT, Bauer J, Pfeiffer RM, Elder DE, Hulley B, Minghetti P, Calista D, Kanetsky PA, Pinkel D, Bastian BC. MC1R germline variants confer risk for BRAF-mutant melanoma. Science. 2006; 313:521–2. [PubMed: 16809487]
17. Fargnoli MC, Pike K, Pfeiffer RM, Tsang S, Rozenblum E, Munroe DJ, Golubeva Y, Calista D, Seidenari S, Massi D, Carli P, Bauer J, Elder DE, Bastian BC, Peris K, Landi MT. MC1R variants increase risk of melanomas harboring BRAF mutations. J Invest Dermatol. 2008; 128:2485–90. [PubMed: 18368129]
18. Hacker E, Hayward NK, Dumenil T, James MR, Whiteman DC. The association between MC1R genotype and BRAF mutation status in cutaneous melanoma: findings from an Australian population. J Invest Dermatol. 2010; 130:241–8. [PubMed: 19571821]
19. Thomas NE, Kanetsky PA, Edmiston SN, Alexander A, Begg CB, Groben PA, Hao H, Busam K, Ollila DW, Berwick M, Conway K. Relationship between germline MC1R variants and BRAF-mutant melanoma in a North Carolina population-based study. J Invest Dermatol. 2010; 130:1463–5. [PubMed: 20043015]
20. Scherer D, Rachakonda PS, Angelini S, Mehnert F, Sucker A, Egberts F, Hauschild A, Hemminki K, Schadendorf D, Kumar R. Association between the germline MC1R variants and somatic BRAF/NRAS mutations in melanoma tumors. J Invest Dermatol. 2010; 130:2844–8. [PubMed: 20720566]
21. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988; 124:869–71. [PubMed: 3377516]
22. Chaudru V, Laud K, Avril MF, Miniere A, Chompret A, Bressac-de Paillerets B, Demenais F. Melanocortin-1 receptor (MC1R) gene variants and dysplastic nevi modify penetrance of CDKN2A mutations in French melanoma-prone pedigrees. Cancer Epidemiol Biomarkers Prev. 2005; 14:2384–90. [PubMed: 16214921]
23. Scherer D, Nagore E, Bermejo JL, Figl A, Botella-Estrada R, Thirumaran RK, Angelini S, Hemminki K, Schadendorf D, Kumar R. Melanocortin receptor 1 variants and melanoma risk: a study of 2 European populations. Int J Cancer. 2009; 125:1868–75. [PubMed: 19585506]
24. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7:248–9. [PubMed: 20354512]
25. Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006; 22:1928–9. [PubMed: 16720584]
26. Arrington JH 3rd, Reed RJ, Ichinose H, Krementz ET. Plantar lentiginous melanoma: a distinctive variant of human cutaneous malignant melanoma. Am J Surg Pathol. 1977; 1:131–43. [PubMed: 602975]
27. Clark WH Jr, Mihm MC Jr. Lentigo maligna and lentigo-maligna melanoma. Am J Pathol. 1969; 55:39–67. [PubMed: 5776171]
28. Clark WH Jr, Elder DE, Van Horn M. The biologic forms of malignant melanoma. Hum Pathol. 1986; 17:443–50. [PubMed: 3699806]
29. Porras BH, Cockerell CJ. Cutaneous malignant melanoma: classification and clinical diagnosis. Semin Cutan Med Surg. 1997; 16:88–96. [PubMed: 9220547]
30. Berwick M, Wiggins C. The current epidemiology of cutaneous malignant melanoma. Front Biosci. 2006; 11:1244–54. [PubMed: 16368510]
31. Valverde P, Healy E, Jackson I, Rees JL, Thody AJ. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nat Genet. 1995; 11:328–30. [PubMed: 7581459]

32. Healy E, Flannagan N, Ray A, Todd C, Jackson IJ, Matthews JN, Birch-Machin MA, Rees JL. Melanocortin-1-receptor gene and sun sensitivity in individuals without red hair. Lancet. 2000; 355:1072–3. [PubMed: 10744096]
33. Orlow I, Tommasi DV, Bloom B, Ostrovnaya I, Cotignola J, Mujumdar U, Busam KJ, Jungbluth AA, Scolyer RA, Thompson JF, Armstrong BK, Berwick M, Thomas NE, Begg CB. Evaluation of the clonal origin of multiple primary melanomas using molecular profiling. J Invest Dermatol. 2009; 129:1972–82. [PubMed: 19282844]
34. Murali R, Goumas C, Kricker A, From L, Busam KJ, Begg CB, Dwyer T, Gruber SB, Kanetsky PA, Orlow I, Rosso S, Thomas NE, Berwick M, Scolyer RA, Armstrong BK. Clinicopathologic features of incident and subsequent tumors in patients with multiple primary cutaneous melanomas. Ann Surg Oncol. 2012; 19:1024–33. [PubMed: 21913010]
35. Titus-Ernstoff L, Perry AE, Spencer SK, Gibson J, Ding J, Cole B, Ernstoff MS. Multiple primary melanoma: two-year results from a population-based study. Arch Dermatol. 2006; 142:433–8. [PubMed: 16618861]
36. Duffy DL, Box NF, Chen W, Palmer JS, Montgomery GW, James MR, Hayward NK, Martin NG, Sturm RA. Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. Hum Mol Genet. 2004; 13:447–61. [PubMed: 14709592]
37. Akey JM, Wang H, Xiong M, Wu H, Liu W, Shriver MD, Jin L. Interaction between the melanocortin-1 receptor and P genes contributes to inter-individual variation in skin pigmentation phenotypes in a Tibetan population. Hum Genet. 2001; 108:516–20. [PubMed: 11499678]
38. Beaumont KA, Newton RA, Smit DJ, Leonard JH, Stow JL, Sturm RA. Altered cell surface expression of human MC1R variant receptor alleles associated with red hair and skin cancer risk. Hum Mol Genet. 2005; 14:2145–54. [PubMed: 15972726]
39. Beaumont KA, Shekar SN, Newton RA, James MR, Stow JL, Duffy DL, Sturm RA. Receptor function, dominant negative activity and phenotype correlations for MC1R variant alleles. Hum Mol Genet. 2007; 16:2249–60. [PubMed: 17616515]
40. Dessinioti C, Antoniou C, Katsambas A, Stratigos AJ. Melanocortin 1 receptor variants: functional role and pigmentary associations. Photochem Photobiol. 2011; 87:978–87. [PubMed: 21749400]
41. Busca R, Abbe P, Mantoux F, Aberdam E, Peyssonnaux C, Eychene A, Ortonne JP, Ballotti R. Ras mediates the cAMP-dependent activation of extracellular signal-regulated kinases (ERKs) in melanocytes. EMBO J. 2000; 19:2900–10. [PubMed: 10856235]
42. Wikberg JE, Muceniece R, Mandrika I, Prusis P, Lindblom J, Post C, Skottner A. New aspects on the melanocortins and their receptors. Pharmacol Res. 2000; 42:393–420. [PubMed: 11023702]
43. Motokawa T, Kato T, Hashimoto Y, Katagiri T. Effect of Val92Met and Arg163Gln variants of the MC1R gene on freckles and solar lentigines in Japanese. Pigment Cell Res. 2007; 20:140–3. [PubMed: 17371441]
44. Elfakir A, Ezzedine K, Latreille J, Ambroisine L, Jdid R, Galan P, Hercberg S, Gruber F, Malvy D, Tschachler E, Guinot C. Functional MC1R-gene variants are associated with increased risk for severe photoaging of facial skin. J Invest Dermatol. 2010; 130:1107–15. [PubMed: 19924138]
45. Scherer D, Bermejo JL, Rudnai P, Gurzau E, Koppova K, Hemminki K, Kumar R. MC1R variants associated susceptibility to basal cell carcinoma of skin: interaction with host factors and XRCC3 polymorphism. Int J Cancer. 2008; 122:1787–93. [PubMed: 18067130]
46. Kvaskoff M, Siskind V, Green AC. Risk factors for lentigo maligna melanoma compared with superficial spreading melanoma: a case-control study in Australia. Arch Dermatol. 2012; 148:164–70. [PubMed: 22004881]
47. Wakamatsu K, Kavanagh R, Kadekaro AL, Terzieva S, Sturm RA, Leachman S, Abdel-Malek Z, Ito S. Diversity of pigmentation in cultured human melanocytes is due to differences in the type as well as quantity of melanin. Pigment Cell Res. 2006; 19:154–62. [PubMed: 16524431]
48. Doyle JR, Fortin JP, Beinborn M, Kopin AS. Selected melanocortin 1 receptor single-nucleotide polymorphisms differentially alter multiple signaling pathways. J Pharmacol Exp Ther. 2012; 342:318–26. [PubMed: 22547573]
49. Thomas NE, Kanetsky PA, Begg CB, Conway K, Berwick M. Melanoma molecular subtypes: unifying and paradoxical results. J Invest Dermatol. 2010; 130:12–4. [PubMed: 20010862]

What's already known about this topic?

The MC1R gene plays a role in pigmentation synthesis, in inflammatory process and activates the mitogen-activated protein kinase pathway.

MC1R variants associated with pigmentation increase the risk of developing melanoma and non melanoma skin cancer.

The p.R163Q variant, not associated with pigmentation, is associated with non melanoma skin cancer in Europeans.

What does this study add?

The p.R163Q variant which is not directly associated to phenotype variation is associated for the first time with the risk of developing lentigo maligna melanoma.

Table 1

Frequency of *MC1R* variants detected in Melanoma patients.

				Total patients (N=1679)
Nucleotide change	**AA change**	**Score Polyphen2[A]**	**Genomic status**	**N (%)**
c.5C>T	**p.A2V**	0.000	Het.	1 (0.1)
			Hom.	-
c.112G>A	p.V38M	0.006	Het.	1 (0.1)
			Hom.	-
c.121T>	**p.S41C**	0.067	Het.	1 (0.1)
			Hom.	-
c.175C>T	**p.V59M**	1.00	Het.	1 (0.1)
			Hom.	-
c.178T>G	p.V60L	0.988	Het.	459 (27.3)
			Hom.	44 (2.6)
c.190G>A	p.A64T	0.988	Het.	1 (0.1)
			Hom.	-
c.248C>T	p.S83L	0.998	Het.	3 (0.2)
			Hom.	-
c.247T>C	p.S83P	0.998	Het.	5 (0.3)
			Hom.	-
c.252C>A	p.D84E*RHC*	0.999	Het.	17 (1.0)
			Hom.	-
c.251C>C	**p.D84H**	1.00	Het.	4 (0.2)
			Hom.	-
c.265G>A	p.G89R	0.737	Het.	1 (0.1)
			Hom.	-
c.274G>A	p.V92M	0.015	Het.	195 (11.6)
			Hom.	2 (0.1)
c.284C>T	p.T95M	0.889	Het.	3 (0.2)
			Hom.	-
c.357C>A	p.V119V	-	Het.	2 (0.1)
			Hom.	-
c.364G>A	p.V122M	0.126	Het.	25 (1.5)
			Hom.	-
c.383T4C	p.M128T	0.235	Het.	3 (0.2)
			Hom.	-
c.425G>A	p.R142H*RHC*	1.000	Het.	54 (3.2)
			Hom.	1 (0.1)
c.424C>A	p.R142S	1.000	Het.	1 (0.1)
			Hom.	-
c.424C>T	p.R142C	1.000	Het.	1 (0.1)
			Hom.	-

				Total patients (N=1679)
Nucleotide change	**AA change**	**Score Polyphen2[A]**	**Genomic status**	**N (%)**
c.434C>T	p.S145F	0.989	Het.	1 (0.1)
			Hom.	-
c.438C>T	**p.A146A**	-	Het.	1 (0.1)
			Hom.	-
c.445G>A	p.A149T	1.000	Het.	2 (0.1)
			Hom.	-
c.446C>T	**p.A149V**	1.00	Het.	1 (0.1)
			Hom.	-
c.451C>T	p.R151C[RHC]	1.000	Het.	139 (8.3)
			Hom.	9 (0.5)
c.464T>C	p.I155T	0.986	Het.	60 (3.6)
			Hom.	4 (0.2)
c.466C>G	p.V156L	0.567	Het.	1 (0.1)
			Hom.	0 (0)
c.467T>C	p.V156A	0.784	Het.	1 (0.1)
			Hom.	-
c.478C>T	p.R160W[RHC]	0.861	Het.	103 (6.1)
			Hom.	1 (0.1)
c.488G>A	p.R163Q	0.004	Het.	69 (4.1)
			Hom.	1 (0.1)
c.504C>T	**p.I168I**	-	Het.	5 (0.3)
			Hom.	-
c.546C>T	p.Y182Y	-	Het.	2 (0.1)
			Hom.	-
c.550G>A	p.D184N	0.001	Het.	1 (0.1)
			Hom.	-
c.586T>C	p.F196L	0.997	Het.	1 (0.1)
			Hom.	-
c.637C>T	p.R213W	0.019	Het.	1 (0.1)
			Hom.	-
c.699G>A	p.Q233Q	-	Het.	30 (1.8)
			Hom.	-
c.741G>A	**p.L247L**	-	Het.	1 (0.1)
			Hom.	-
c.766C>T	p.P256S	1.000	Het.	1 (0.1)
			Hom.	-
c.788T>C	**p.L263P**	1.00	Het.	1 (0.1)
			Hom.	-
c.792C>T	p.I264I	-	Het.	1 (0.1)
			Hom.	-
c.793G>A	p.V265I	0.067	Het.	1 (0.1)

				Total patients (N=1679)
Nucleotide change	**AA change**	**Score Polyphen2*A***	**Genomic status**	**N (%)**
			Hom.	-
c.813C>T	p.P271P	-	Het.	1 (0.1)
			Hom.	-
c.814A>G	p.T272A	0.006	Het.	2 (0.1)
			Hom.	-
c.815C>T	**p.T272M**	0.974	Het.	1(0.1)
			Hom.	-
c.815C>A	p.T272K	0.944	Het.	2 (0.1)
			Hom.	-
c.835A>G	**p.N279D**	0.979	Het.	1 (0.1)
			Hom.	-
c.850C>T	**p.L284F**	0.965	Het.	1 (0.1)
			Hom.	-
g.860T>G	p.I287S	0.996	het.	1 (0.1)
			hom.	-
c.861C>G	p.I287M	0.999	het.	1 (0.1)
			hom.	-
g.880G>C	p.D294H*RHC*	1.000	het.	151 (9.0)
			hom.	7 (0.4)
c.892 C>T	**p.R298R**		het.	1 (0.1)
			hom.	-
c.923C>T	p.T308M	0.979	het.	1 (0.1)
			hom.	-
g.942A>G	p.T314T	-	het.	287 (17.1)
			hom.	8 (0.5)
g.948C>T	p.S316S	-	het.	2 (0.1)
			hom.	-

Novel variants are indicated in bold.

A In-silico impact prediction of each non-synonymous variants on the structure and MC1R function (values close to 0.000: benign; values close to 1.00: damaging).

RHC Variant associated to Red hair colour phenotype. Het: variant in heterozygosis. Hom: Variant in homozygosis.

Table 2

Analysis of *MC1R* and phenotypical traits.

A. Association of recurrent MC1R with phenotypical traits[B]					
Hair colour		**Brown/Black**	**Red**		
***MC1R* Variant**	**Genotype**	**N (%)**	**N (%)**	**OR (95% CI)**[A]	**P-value**
p.R142H					
	G/G	1168 (97.9)	58 (78.4)	1.00	
	G/A	25 (2.1)	15 (20.3)	12.40 (6.18–24.89)	<0.0001[C]
	A/A	0 (0)	1 (1.4)	NA	
p.R151C					
	C/C	1118 (93.7)	41 (55.4)	1.00	
	C/T	75 (6.3)	28 (37.8)	10.27 (6.01–17.56)	<0.0001[C]
	T/T	0 (0)	5 (6.8)	NA	
p.R160W					
	C/C	1132 (94.9)	58 (78.4)	1.00	
	C/T	61 (5.1)	16 (21.6)	5.2 (2.7–9.4)	<0.0001[C]
	T/T	0 (0)	0 (0)	NA	
p.D294H					
	G/G	1102 (92.4)	43 (58.1)	1.00	
	G/C	91 (7.6)	25 (33.8)	6.96 (4.06–11.93)	<0.0001[C]
	C/C	0 (0)	6 (8.1)	NA	
Skin phototype		**III–IV**	**I–II**		
***MC1R* Variant**	**Genotype**	**N (%)**	**N (%)**	**OR (95% CI)**[A]	**P-value**
p.R142H					
	G/G	930 (97.6)	608 (95.3)	1.00	
	G/A	23 (2.4)	29 (4.5)	1.95 (1.12–3.41)	0.027[C]
	A/A	0 (0)	1 (0.2)	NA	
p.R151C					
	C/C	897 (94.1)	552 (86.5)	1.00	
	C/T	55 (5.8)	79 (12.4)	2.36 (1.65–3.39)	<0.0001[C]

A. Association of recurrent MC1R with phenotypical traits[B]

Hair colour		**Brown/Black**	**Red**		
***MC1R* Variant**	**Genotype**	**N (%)**	**N (%)**	**OR (95% CI)**[A]	**P-value**
	T/T	1 (0.1)	7 (1.1)	10.71 (1.31–87.42)	
p.R160W					
	C/C	906 (95.1)	584 (91.5)	1.00	
	C/T	47 (4.9)	53 (8.3)	1.73 (1.15–2.60)	0.0018[C]
	T/T	0 (0)	1 (0.2)	NA	
p.D294H					
	G/G	883 (92.7)	553 (86.7)	1.00	
	G/C	70 (7.3)	78 (12.2)	1.76 (1.25–2.48)	<0.0001[C]
	C/C	0 (0)	7 (1.1)	NA	
Eye colour		**Dark**	**Green/Blue**		
***MC1R* Variant**	**Genotype**	**N (%)**	**N (%)**	**OR (95% CI)**[A]	**P-value**
p.R142H					
	G/G	896 (97.6)	571 (95.2)	1.00	
	G/A-A/A	22 (2.4)	29 (4.8)	2.07 (1.18-3-65)	0.011[D]

B. Association of number of *MC1R* variants with phenotypical traits

	Total number of variants				
	0 Var (%)[X]	***1 Var (%)***[X]	***≥2 Var (%)***[X]	***TOTAL (%)***	**P-value**
Hair colour					
black/brown	565 (47.4)	439 (36.8)	189 (15.8)	1193 (100)	
red	4 (5.4)	12 (16.2)	58 (78.4)	74 (100)	<0.0000
	Total number of variants				
	0 Var (%)[X]	***1 Var (%)***[X]	***≥2 Var (%)***[X]	***TOTAL (%)***	
Photoype					
I–II	219 (34.3)	235 (36.8)	184 (28.8)	638 (100)	
III–IV	472 (49.5)	338 (35.5)	143 (15.0)	953 (100)	<0.0000
Eye colour					
fair	268 (44.7)	207 (34.5)	125 (20.8)	600 (100)	
dark	392 (42.7)	336 (36.6)	190 (20.7)	918 (100)	n.s

B. Association of number of *MC1R* variants with phenotypical traits

	Total number of variants				
	0 Var (%) [X]	***1 Var (%)*** [X]	***≥2 Var (%)*** [X]	***TOTAL (%)***	**P-value**
	Number of non Red hair colour (r) variants				
	0 Var (%) [X]	***1 Var (%)*** [X]	***≥2 Var (%)*** [X]	***TOTAL (%)***	**P-value**
Hair colour					
black/brown	716 (60)	392 (32.9)	85 (7.1)	1193 (100)	
red	53 (71.6)	19 (25.7)	2 (2.7)	74 (100)	n.s
Photoype					
I–II	354 (55.5)	231 (36.2)	53 (8.3)	638 (100)	
III–IV	596 (62.5)	293 (30.7)	64 (6.7)	953 (100)	0.041
Eye colour					
fair	361 (60.2)	199 (33.2)	40 (6.7)	600 (100)	
dark	544 (59.3)	299 (32.6)	75 (8.2)	918 (100)	n.s
	Number of Red hair colour (R) variants				
	0 Var (%) [X]	***1 Var (%)*** [X]	***≥2 Var (%)*** [X]	***TOTAL (%)***	**P-value**
Hair colour					
black/brown	939 (78.7)	249 (20.9)	5 (0.4)	1193 (100)	
red	8 (10.8)	26 (35.1)	40 (54.1)	74 (100)	<0.0000
Photoype					
I–II	417 (65.4)	174 (27.3)	47 (7.4)	638 (100)	
III–IV	756 (79.3)	188 (19.7)	9 (0.9)	953 (100)	<0.0000
Eye colour					
fair	442 (73.7)	137 (22.8)	21 (3.5)	600 (100)	
dark	680 (74.1)	205 (22.3)	33 (3.6)	918 (100)	n.s

[B] Analysis was performed for each recurrent variant (p.V60L, p.V92M, p.D294H, p.R151C, p.R160W, p.R163Q, p.R142H, p.I155T, p.V122M and p.D84E): Only MC1R variants with statistically significant P-values are shown.

[A] ORs are adjusted by age of onset, gender and hospital of recruitment. Model of heritance was chosen according to the AIC and BIC values:

[C] :Codominant model;

[D] :Dominant model.

[X] Number and frequency of patients. NA: not analyzed. n.s: P-values not statistically significant

Table 3

Number of variants and histopathological subtype of melanoma.
Analysis was performed separately by MM patient subtype. In the MPM subgroup only the first tumours were included in the analysis.

	Total *MC1R* Variants				
	0 (%)	**1 (%)**	**≥2 (%)**	**Total (%)**	**P-value**
LMM	57 (41.9)	53 (39.0)	26 (19.1)	136 (100)	0.028
SSM	503 (42.4)	433 (35.5)	251(21.1)	1187 (100)	n.s.
NM	122 (45.4)	96 (35.7)	51 (19)	269 (100)	n.s.
ALM	54 (62.1)	23 (26.4)	10 (11.5)	87 (100)	n.s.
	Number or non Red hair colour variants				
	0 (%)	**1 (%)**	**≥2 (%)**	**Total (%)**	**P-value**
LMM	82 (60.3)	47 (34.6)	7 (5.1)	136 (100)	0.382
SSM	698 (58.8)	401 (33.8)	88 (7.4)	1187 (100)	n.s.
NM	163 (60.0)	87 (32.3)	19(7.1)	269 (100)	n.s.
ALM	61 (70.1)	19 (21.8)	7 (8.0)	87 (100)	n.s.
	Number Red hair colour variants				
	0 (%)	**1 (%)**	**≥2 (%)**	**Total (%)**	**P-value**
LMM	98 (72.1)	32 (23.5)	6 (.4.4)	136 (100)	0.035
SSM	875 (73.7)	264 (22.2)	48 (4.0)	1187 (100)	n.s.
NM	199 (74.0)	67 (24.9)	3 (1.1)	269 (100)	n.s.
ALM	75 (86.2)	11 (12.6)	1 (1.1)	87(100)	n.s.

LMM: Lentigo maligna melanoma, SSM: Superficial spreading melanoma, NM: Nodular melanoma, ALM: Acral lentiginous melanoma.

n.s. not significant

Table 4

Association of p.R163Q and Lentigo Maligna Melanoma tumours (LMM).

***MC1R* Variant p.R163Q**	**Genotype**	**No LMM N (%)[1]**	**LMM N (%)[2]**	**OR (95% CI)[A]**	**P-value**
	G/G	1466 (96.1)	143 (92.9)	1.00	
	G/A-A/A	59 (3.9)	11 (7.1)	2.16 (1.07–4.37)	0.044 [D]

[1] Number of patients who did not develop any LMM.

[2] Number of patients who develop at least one tumour classified as LMM.

[A] ORs Adjusted by age of onset, gender, number of MM, number of MC1R variants, skin phototype, hair colour and Hospital of recruitment. Model of heritance was chosen according the AIC and BIC values:

[D] Dominant model.