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Risk factors for keratinocyte skin cancer in patients diagnosed with melanoma, a large retrospective study.

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Abstract

BACKGROUND: Melanoma survivors are at an increased **risk** of developing other malignancies, including **keratinocyte skin cancer** (KSC). While it is known that many **risk factors** for **melanoma** also impact **risk** of KSC in the general population, no previous **study** has investigated **risk factors** for KSC development in **melanoma patients**.

OBJECTIVE: We assessed associations of personal and clinical characteristics, including **skin** phenotype and variations in the melanocortin 1 receptor (MC1R) gene, with KSC **risk** in **melanoma patients**.

PATIENTS AND METHODS: We used prospective follow-up information on 1200 **patients** treated for **melanoma** at the Instituto Valenciano de Oncología, Spain, between 2000 and 2011. We computed hazard ratios and 95% confidence intervals (CIs) for the association of clinical, personal and genetic characteristics with **risk** of KSC, squamous cell carcinoma (SCC), or basal cell carcinoma (BCC) from Cox proportional hazard models. Five-year cumulative incidence based on competing **risk** models of SCC, BCC or KSC overall was computed using multivariate subdistribution hazard models. To assess predictive performance of the models, we computed areas under the receiver-operating characteristic curves (AUCs, discriminatory power) using cross-validation.

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Authors' contribution to this study

Pablo Espinosa: literature search, data collection, and writing. Ruth M. Pfeiffer: study design, figures, data analysis, data interpretation, and writing. Zaida García-Casado: data collection and writing. Celia Requena: data collection. Maria Teresa Landi: data interpretation and critical revision of the manuscript. Rajiv Kumar: data collection, data interpretation, and writing. Eduardo Nagore: literature search, conception and design of the study, data collection, data interpretation, and writing. All the authors gave their final approval to the submitted version.

Conflict of interest statement

None exists.

RESULTS: Median follow-up was 57.2 months; a KSC was detected in 163 **patients** (13.6%). In multivariable Cox models, age, sex, sunburns, chronic sun exposure, past personal history of non-melanoma skin cancer or other non-cutaneous neoplasia, and the MC1R variants p.D294H and p.R163Q were significantly associated with KSC **risk**. A cumulative incidence model including age, sex, personal history of KSC, and of other non-cutaneous neoplasia had an AUC of 0.76 (95% CI: 0.71–0.80). When p.D294H and p.R163Q variants were added to the model, the AUC increased to 0.81 (95% CI: 0.77–0.84) (p-value for difference <0.0001).

CONCLUSIONS: In addition to age, sex, **skin** characteristics, and sun exposure, p.R163Q and p.D294H MC1R variants significantly increased KSC **risk** among **melanoma patients**. Our findings may help identify **patients** who could benefit most from preventive measures.

Keywords

Keratinocyte skin cancer; MC1R; Melanoma; Non-melanoma skin cancer; Risk factors; Second malignancy

1. Introduction

Although malignant melanoma remains the major cause of death associated with skin cancers, public awareness, early detection and improved treatment strategies have markedly prolonged patient survival. Increased incidence and improved survival have led to an ever increasing number of melanoma survivors. However, like survivors of other cancers, patients who survive melanoma are also at an increased risk of developing second primary malignancies, which include second melanomas and keratinocyte skin cancers (KSCs) [1], [2], [3], [4].

Considering the morbidity caused by KSC in melanoma survivors and the added burden to healthcare systems, it is important to identify factors associated with increased risk for such malignancies for targeted prevention efforts. We hypothesised that risk factors associated with both primary melanoma and KSC also increase KSC risk in melanoma survivors. Factors associated with both risk of melanoma and risk of KSC include susceptible skin phototype with propensity to sunburn and sun exposure. Genetic variations in low-penetrant pigmentation genes that are major determinants of predisposing phenotypes like skin and hair colour are also known risk factors for both melanoma and KSC [5], [6]. Variants in the melanocortin 1 receptor (*MC1R*) gene are among the most important genetic determinants of high-risk phenotypes like fair skin and red hair and consequently increased risk of skin cancers in general [7], [8], [9], [10], [11], [12], [13], [14], [15].

Despite known risk of KSC in patients who survived melanoma, no prospective study to date has assessed risk factors in detail. Thus, we investigated the association of personal, clinical and genetic factors with risk of developing of KSC in melanoma patients in a cohort of 1200 melanoma patients followed for over 10 years. We also estimated cumulative incidence of KSC risk to stratify patients for increased surveillance and preventative measures.

2. Patients and methods

Patients with cutaneous melanoma in this study include individuals who had been treated at the Instituto Valenciano de Oncología between 1st January 2000 and 31st December 2011. At the end of this period, the database contained information for 1792 incident and prevalent patients. A wide range of clinical, epidemiological and histological variables evaluated by dermatologists with specialised training in melanoma management were collected, as described in detail previously [16].

In brief, all patients were regularly followed-up at the Instituto Valenciano de Oncología by a dermatologist (E.N. or C.R.) devoted to melanoma management according to current protocols. Follow-up visits were scheduled at least twice per year for the first 3 years after melanoma diagnosis and yearly thereafter and also included a whole-skin clinical examination. At each visit, routine anamnesis included questions about any health event and, particularly, about cutaneous lesions treated in other centers. When appropriate, the diagnosis of squamous cell carcinoma (SCC) or basal cell carcinoma (BCC) was assessed by pathological report. KSCs (BCC or SCC) diagnosed after the melanoma were histologically confirmed in all patients. For the purpose of this analysis, we also included synchronous tumours, defined as skin tumours diagnosed either simultaneously or within 3 months of melanoma diagnosis.

The study population was restricted to patients 18 years or older at the time of melanoma diagnosis. Patients with xeroderma pigmentosum, mucosal melanomas or with unknown primary were excluded. One patient, for whom information about BCC and SCC after melanoma diagnosis was not available, was also excluded. The final analytic population in this study comprised 1200 individuals (Table 1 and Supplementary Table 1).

2.1. Genotyping

MC1R variants were genotyped by direct DNA sequencing according to previously described methods [17]. Sequence analysis was performed using the ABI Prism system (Applied Biosystems, Foster City, CA) with the Big Dye Terminator Cycle Kit and the ABI 3700 automated sequencer.

2.2. Statistical analysis

Associations of patient characteristics and melanoma features with BCC or SCC risk were assessed using chi-square and Fisher exact tests. Cox proportional hazard regression models were used to estimate hazard ratios and 95% confidence intervals (CIs) as measures of association between risk factors and incidence of BCC or SCC. Follow-up time started at time of primary melanoma diagnosis and ended at the time of diagnosis of a first BCC or SCC or censoring, whichever occurred first. Censoring events were death or end of follow-up. The development of other neoplasias, including subsequent melanomas, was not considered as censoring events in the analysis. For the BCC analysis, we did not censor a person when he or she developed a prior SCC and vice versa. All models were adjusted for age at diagnosis (used in the categories <43.7, 43.7–56.8, 56.8–68.5, and ≥68.5 based on population quartiles). In addition to evaluating BCC and SCC incidence separately, we also

combined BCC and SCC into a composite end-point, defined as the first occurrence of BCC or SCC. In sensitivity analysis, we used age as the time metric for the Cox regression models and started follow-up at age of melanoma diagnosis to account for left truncation.

The following factors were evaluated as potential risk factors: sex; histological subtype of melanoma (lentigo maligna melanoma [LMM], superficial spreading melanoma [SSM], nodular melanoma, acral lentiginous melanoma and others/non-specified) and location (head/neck, upper extremities, trunk, lower extremities, and acral); past personal history of KSC (BCC and/or SCC) and other non-cutaneous malignancies; history of non-cutaneous malignancies in first-degree relatives (yes/no); phototype (I–II versus III–IV); hair colour (black/brown, blond, and red); eye colour (dark/fair); number of nevi (<50 versus ≥50); presence of solar lentigines (yes/no) and actinic keratosis (AK) (yes/no); a history of chronic work-related sun exposure for at least 20 years (yes/no); history of more than five severe sunburns (with pain for at least 48 h or blistering, yes/no); and smoking (non-smoker, smoker with ≤20 pack-years, and smoker with >20 pack-years). We also assessed the following genetic risk factors: carrying zero, one or more than one non-synonymous *MC1R* variant; carrying any R variant (yes/no, based on the presence of any of the following variants: p.D84E, p.R142H, p.R151C, p.R160W and p.D294H); and carrying each of the above-mentioned R variants as well as p.R163Q variant (yes/no) separately. p.R163Q was included because it has been previously shown to increase BCC risk [14].

We computed 5-year cumulative incidence based on competing risk models of SCC, BCC or the combined end-point using multivariable subdistribution hazard models [18]. Time since melanoma diagnosis was used as the time scale for this analysis. We fitted two models to each outcome, one based on clinical risk factors alone, which we had verified that were associated with either the death or the outcome in Cox regression models, and the second that included genetic variants that were significantly associated with either the death or the outcome in addition to the clinical risk factors. We also computed cumulative incidence based on a simple model that included age, sex, personal history of KSC, and personal history of other non-cutaneous malignancy, the R163Q and p.D294H variants. We then compared the predictive ability of each model, as assessed by the area under the receiver-operating characteristic (ROC) curve (AUC). To obtain unbiased estimates of the AUC value for each model, we used leave one out cross-validation [19] and compared the AUC values using the de-Long test [20]. AUC CIs and p-values based on a stratified bootstrap with 2000 bootstrap replications yielded similar results (data not shown).

P values <0.05 were considered statistically significant. All analyses were carried out with the SAS 9.1 statistical software package and the packages pROC and cmprsk in R [21].

3. Results

The investigated population included 1200 patients with median age at diagnosis of the first primary melanoma of 56.8 years (range 18.4–96.1 years). The characteristics of the population are detailed in Table 1 and Supplementary Table 1 (by groups defined by the development of KSC, SCC or BCC). A 50.7% of the patients were female. Most patients (61.2%) had skin phototype III–V, dark hair (73.0%) and dark eyes (59.4%); 14.0% of

patients reported intense sun exposure at work (for >20 years) and 17.9% recalled more than five severe lifetime sunburns. Furthermore, 82.1% of patients had solar lentigines. Only 74 patients (6.2%) reported a family history of melanoma.

SSM was the most common histological type (60.6%), and trunk was the most common site of primary melanoma (38.0%), followed by head and neck (20.7%) and the lower extremities (18.2%). Of 958 (79.8%) patients who had genotype data, 32.5% carried at least one R variant in *MC1R*.

Median follow-up time of the cohort was 57.2 months after melanoma diagnosis (range 0–167.9 months). During the follow-up, KSC (BCC or SCC) was detected in 163 patients (13.6%), 51 of which were diagnosed in the first 6 months after melanoma diagnosis (synchronous tumours). There were 184 deaths during follow-up, with 147 occurring during the first 5 years after melanoma diagnosis. Cumulative incidences at 12, 24 and 60 months were 5.9%, 7.7% and 10.8%, respectively. Most patients (69.8%) were diagnosed with stage I/II melanoma, while 14.4% had had III and only 0.6% had stage IV melanoma. Two individuals (0.17%) had missing stage information and 15.1% were diagnosed with in situ melanoma.

In age-adjusted Cox regression models, male sex; red hair colour; sunburns; smoking of >20 pack-years; presence of AK; a previous KSC (BCC, SCC or both); personal history of other non-cutaneous malignancy; and two variants of *MC1R*, p.D294H and p.R163Q, were associated with statistically significant increased risk of developing an KSC (Table 2). In our dataset, smoking was significantly although weakly correlated (Spearman rank correlation $p < 0.001$) with male sex, presence of solar lentigines, a history of chronic work-related sun exposure for at least 20 years, and history of more than five severe sunburns.

In multivariable models, age, male sex, sunburns, chronic sun exposure, personal history of KSC or other non-cutaneous malignancy and the variants p.D294H and p.R163Q remained associated with the risk of KSC (Table 3).

We also analysed BCC and SCC separately. In age-adjusted Cox models, male sex, sunburns, personal history of KSC and harboring at least one RHC variant and the variant p.R163Q of *MC1R* increased BCC risk (Table 2). In an age-adjusted multivariable model, the risk of SCC was associated with red hair colour, light eye colour and the presence of AK.

We then fitted cumulative incidence models including the risk factors identified below to SCC or BCC or KSC that occurred within 5 years of the primary melanoma diagnosis. Death was treated as a competing risk in those models. For BCC, the cumulative incidence model with only clinical characteristics included age, sex, past history of sunburns, melanoma anatomical site, LMM subtype, presence of AK, a personal history of BCC, a personal history of other non-cutaneous malignancy and stage (model 1). The AUC estimate for this model was 0.75 (95% CI: 0.69–0.79). A model that in addition included p.R163Q and p.D294H (both coded with a trend in the number of minor alleles and excluding individuals with missing genotype, model 2) had an AUC of 0.78 (95% CI: 0.72–0.82), which was significantly higher than the AUC of model 1 (p -value <0.0001). For SCC, the AUC estimate for the model with clinical characteristics only (model 1) that included age,

sex, past history of sunburns, melanoma site, smoking, LMM subtype, AK, red hair colour, and eye colour was 0.67 (95% CI: 0.50–0.84) and for a model that in addition included p.R163Q, the AUC was 0.78 (95% CI: 0.65–0.91), (p-value for difference 0.01); however, this evaluation was based on only 16 SCC cases that occurred within 5 years of melanoma diagnosis. When SCC and BCC were combined into a composite outcome, the AUC value for a model with clinical characteristics was 0.76 (95% CI: 0.71–0.80) and when we added p.R163Q to the model, the AUC was significantly higher, 0.81 (95% CI: 0.77–0.84) (p-value for difference <0.0001). The ROC curves for these two models are shown in Fig. 1. The AUC value for the composite outcome for a model including age, sex, personal history of KSC, and personal history of other non-cutaneous malignancy (AUC = 0.71, 95% CI: 0.66–0.77) was significantly lower than that of a model that in addition to these variables also included the *MC1R* variants p.D294H and p.R163Q variants (AUC = 0.78, 95% CI: 0.74–0.83; p-value for difference <0.0001). Figure 2 shows the cumulative incidence estimates for that latter model for everybody in the study cohort who had complete covariate information. While most individuals had cumulative 5-year incidence below 20%, for some individuals cumulative incidence was >50% 24 months after melanoma diagnosis.

4. Discussion

It is well known that patients with melanoma are at an elevated risk of developing non-melanoma skin cancers; [22] however, associated factors have not been comprehensively investigated. We thus studied cumulative incidence of KSC and related risk factors in a large cohort of melanoma patients. We also estimated the role of *MC1R* variants in the development of KSC, particularly BCC, in patients diagnosed with cutaneous melanoma. In our population, the estimated cumulative incidence of KSC in melanoma survivors at 5 years from the melanoma diagnosis was 11%, which is much higher than the reported incidence of 45 new cases per year per 100,000 inhabitants in the region of Valencia area of Spain [23]. While a small proportion of this incidence difference could be due to different age and sex distribution between the general population and our cohort of melanoma patients, the main reasons for the difference are likely to be: 1. each subject in our study was diagnosed with a previous melanoma, and as many risk factors for melanoma are shared with non-melanoma skin cancer, these individuals are at higher baseline risk than the general population; 2. these patients had been under close clinical follow-up during the first 5 years after the melanoma diagnosis, which may have led to higher diagnosis rate; and 3. typically, incidence of BCC and SCC in cancer registries is underestimated because many of these cancers are diagnosed and treated in private practices and are not reported in cancer registry accruing data from hospital discharge records.

The main known environmental factor associated with SCC and BCC risk is sun exposure [24]. However, the pattern of sun exposure associated with each cancer type differs with chronic sun exposure associated with SCC and acute damaging sun exposure with BCC. Accordingly, in our melanoma patients, a personal history of sunburns was a risk factor for BCC development but not for SCC. In contrast, AK, a well-recognised clinical marker of severe chronic sun damage and precursor of SCC, was significantly associated with SCC risk but not with BCC risk. Pigmentation characteristics such as hair and eye colour were only significantly associated with SCC risk, highlighting the importance of the interplay

between fair skin and chronic sun exposure in development of this tumour [25]. All these findings together with the absence of association with nevi count and the higher frequency of LMM type (although without statistical significance in Cox regression models) in patients who developed KSC seem to support that the profile of melanoma patients developing subsequent KSC falls into the cumulative sun damage pathway previously proposed as one of the divergent pathways of melanoma development [26]. The absence of risk associated to higher number of melanocytic nevi merits further discussion as the BCC risk has been shown to be higher in patients with increasing number of melanocytic nevi [27]. In our opinion, the most plausible explanation is that melanoma patients do not represent the general population; they have typically a higher number of nevi at baseline, and thus, there is less variability across melanoma cases who develop or do not develop KSC. Since nevi frequency changes with age, we stratified subjects based on median age, but observed no association between nevi and KSC risk in any age strata (data not shown). In Cox regression models adjusted only for age, the number of nevi were not associated with 5-year risk of KSC. However, as can be seen in Supplementary Table 1, the percentage of patients with >20 nevi was larger in those who did not develop a KSC, again suggesting a sun-related pathway more than a nevi-related pathway in the development of KSC in melanoma patients.

Risk factors for BCC were advanced age, severe sunburns, outdoors working and the diagnosis of a prior KSC [28]. Age, light eye colour and the presence of AK were risk factors for SCC. Two *MC1R* variants, p.D294H and p.R163Q, were also significantly associated with a two-fold risk of developing a KSC in melanoma patients. p.D294H, the most common variant in the study population [13], is also associated with high-risk phenotypes (red hair, low skin pigmentation and skin phototype) and it confers the highest risk for melanoma development among all *MC1R* variants in this population [13]. p.R163Q has been shown to be involved in the predisposition to BCC and not melanoma except for lentigo maligna/LMM subtype [14], [29]. It is also possible that this variant influences pathways other than those involved in melanin synthesis. For example, p.R163Q has been shown to result in a selective decrease in MAPK activation [30]. Furthermore, this variant has also been shown to impact risk of freckles and solar lentigines, both associated with UV exposure [31]. Non-pigmentary functions of *MC1R* include regulation of cytokines and their receptors in immune and inflammatory responses through modulation of nuclear factor-kappa B. The effect of *MC1R* variants on the immune regulation and anti-inflammatory actions of alpha melanocyte-stimulating hormone (α -MSH) could also cause increased susceptibility. Of note, KSC, particularly cutaneous SCC, is a common malignancy in melanoma patients treated with serine/threonine-protein kinase B-Raf (BRAF) inhibitors [32].

While models that predicted KSC development based on clinical information alone had good discriminating performance, adding genetic information significantly improved predictive performance in our population. As we show results from models (available upon request) based on objectively measureable variables (age, sex, personal history of KSC, personal history of other non-cutaneous malignancies, and p.D294H and p.R163Q variants), they could be easily validated and tested in other populations.

Limitations of our study include possible recall bias for all variables related to sun exposure. Also, the limited follow-up time did not allow obtaining accurate estimations for long-term risks (e.g. 10 years). Missing information on some factors caused us to restrict the multivariable analysis to 843 of the total of 1200 patients. The number of SCC diagnoses was small; thus, any SCC results should be interpreted with caution, including AUC comparisons for the risk prediction model.

Strengths of our study are the large population of melanoma patients, prospectively collected data, examined and treated at a single institution, detailed personal characteristics and careful assessment of BCC and SCC based on pathological or medical reports. In addition, we provide for the first time cumulative incidence values for KSC that could be relevant for clinical management of the patients.

In summary, we found that in addition to age, sex and patient characteristics, p.R163Q and p.D294H *MC1R* variants were significantly associated with KSC risk among melanoma patients. Our findings may help identify patients at elevated risk who could benefit from preventive measures, particularly education about self-examination and sun protection, as well as more intense medical follow-up.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- [1]. Crocetti E, Guzzinati S, Pad E, Falcini F, Zanetti R, Vercelli M. et al. The risk of developing a second, different, cancer among 14 560 survivors of malignant cutaneous melanoma: a study by AIRTUM (the Italian Network of Cancer Registries). *Melanoma Res* 2008;18(3):230–4. [PubMed: 18477899]
- [2]. Levi F, La Vecchia C, Randimbison L, Te VC, Erler G. Incidence of invasive cancers following cutaneous malignant melanoma. *Int J Cancer J Int du Cancer* 1997;72(5):776–9.
- [3]. Spanogle JP, Clarke CA, Aroner S, Swelter SM. Risk of second primary malignancies following cutaneous melanoma diagnosis: a population-based study. *J Am Acad Dermatol* 2010;62(5):757–67. [PubMed: 20223559]
- [4]. van der Leest RJ, Flohil SC, Arends LR, de Vries E, Nijsten T. Risk of subsequent cutaneous malignancy in patients with prior melanoma: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol* 2015;29(6): 1053–62. [PubMed: 25491923]
- [5]. Scherer D, Kumar R. Genetics of pigmentation in skin cancer-a review. *Mutation Res* 2010;705(2): 141–53. [PubMed: 20601102]
- [6]. Yokoyama S, Woods SL, Boyle GM, Aoude LG, MacGregor S, Zismann V, et al. A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. *Nature* 2011; 480(7375):99–103. [PubMed: 22080950]
- [7]. Kanetsky PA, Panossian S, Elder DE, Guerry D, Ming ME, Schuchter L. et al. Does MC1R genotype convey information about melanoma risk beyond risk phenotypes? *Cancer* 2010; 116(10):2416–28. [PubMed: 20301115]

- [8]. Raimondi S, Sera F, Gandini S, Iodice S, Caini S, Maisonneuve P, et al. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. *Int J Cancer J Int du Cancer* 2008; 122(12): 2753–60.
- [9]. Kennedy C, Ier Huurnc J, Berkhouli M, Gruis N, Bastiacns M, Bergman W, et al. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. *J Investig Dermatol* 2001; 117(2):294–300. [PubMed: 11511307]
- [10]. Matichard E, Verpillat P, Meziani R, Gerard B, Descamps V, Legroux E, et al. Melanocortin 1 receptor (MC1R) gene variants may increase the risk of melanoma in France independently of clinical risk factors and UV exposure. *J Medical Genetics* 2004; 41(2):e13.
- [11]. Landi MT, Kanetsky PA, Tsang S, Gold B, Munroe D, Rebbeck T, et al. MC1R, ASIP, and DNA repair in sporadic and familial melanoma in a Mediterranean population. *J Nall Cancer Inst* 2005;97(13):998–1007.
- [12]. Hoiom V, Tuominen R, Kaller M, Linden D, Ahmadian A, Mansson-Brahme E, et al. MC1R variation and melanoma risk in the Swedish population in relation to clinical and pathological parameters. *Pigm Cell Melanoma Res* 2009;22(2):196–204.
- [13]. Scherer D, Nagore E, Bermejo JL, Figl A, Botella-Estrada R, Thirumaran RK, et al. Melanocortin receptor 1 variants and melanoma risk: a study of 2 European populations. *Int J Cancer J Int du Cancer* 2009; 125(8): 1868–75.
- [14]. Scherer D, Bermejo JL, Rudnai P, Gurzau E, Koppova K, Ilemminki K, et al. MC1R variants susceptibility to basal cell carcinoma of skin: interaction with host factors and XRCC3 polymorphism. *Int J Cancer J Int du Cancer* 2008;122(8): 1787–93.
- [15]. Bastiacns MT, ter Huume JA, Kielich C, Gruis NA, Westendorp RG, Vermeer BJ, et al. Melanocortin-1 receptor gene variants determine the risk of nonmelanoma skin cancer independently of fair skin and red hair. *Am J Human Genetics* 2001; 68(4):884–94. [PubMed: 11254446]
- [16]. Nagore E, Botella-Estrada R, Requena C, Serra-Guillen C, Martorell A, Hueso L, et al. Clinical and epidemiologic profile of melanoma patients according to sun exposure of the tumor site. *Actas dermo Sifiliogr* 2009;100(3):205–11.
- [17]. Garcia-Casado Z, Traves V, Banuls J, Niveiro M, Gimeno-Carpio E, Jimenez-Sanchez AI, et al. BRAE, NRAS and MC1R status in a prospective series of primary cutaneous melanoma. *Br J Dermatol* 2015; 172(4): 1128–31. [PubMed: 25385688]
- [18]. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Statist Assoc* 1999;94(446): 496–509.
- [19]. Molinaro AM, Simon R, PfeilYer RM. Prediction error estimation: a comparison of resampling methods. *Bioinformatics* 2005; 21(15):3301–7. [PubMed: 15905277]
- [20]. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988;44(3): 837–45. [PubMed: 3203132]
- [21]. Ihaka R, Gentleman RR. A language for data analysis and graphics. *J Comput Graph Stat* 1996;5:299–314.
- [22]. Caini S, Boniol M, Botteri E, Tosti G, Bazolli B, Russell-Edu W, et al. The risk of developing a second primary cancer in melanoma patients: a comprehensive review of the literature and meta-analysis. *J Dermatol Science* 2014;75(1):3–9.
- [23]. Vilar-Coromina N, Miro-Queralt J, Cano-Bautista A, Vilardell-Gil L, Torres Babie P, Marcos-Gragera R. Non-melanoma skin cancer: incidence time trends analysis in Girona, Spain, 1994–2007. *Medicina Clinica* 2011;137(4): 145–51. [PubMed: 21601891]
- [24]. Rosso S, Zanetti R, Martinez C, Tormo MJ, Schraub S, Sancho-Garnier H, et al. The multicentre south European study ‘Helios’. II: different sun exposure patterns in the aetiology of basal cell and squamous cell carcinomas of the skin. *Br J Cancer* 1996; 73(11): 1447–54. [PubMed: 8645596]
- [25]. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. *N Engl J Med* 2001 ;344(13):975–83. [PubMed: 11274625]

- [26]. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. *J Natl Cancer Inst* 2003; 95(11):806–12. [PubMed: 12783935]
- [27]. Khalesi M, Whiteman DC, Tran B, Kimlin MG, Olsen CM, Neale RE. A meta-analysis of pigmentary characteristics, sun sensitivity, freckling and melanocytic nevi and risk of basal cell carcinoma of the skin. *Cancer Epidemiol* 2013;37(5):534–43. [PubMed: 23849507]
- [28]. Marks R, Rennie G, Selwood T. The relationship of basal cell carcinomas and squamous cell carcinomas to solar keratoses. *Arch Dermatol* 1988; 124(7): 1039–42. [PubMed: 3389848]
- [29]. Puig-Butille JA, Carrera C, Kumar R, Garcia-Casado Z, Badenas C, Aguilera P, et al. Distribution of MC1R variants among melanoma subtypes: p.R163Q is associated with lentigo maligna melanoma in a Mediterranean population. *Br J Dermatol* 2013; 169(4):804–11. [PubMed: 23647022]
- [30]. 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(2):318–26. [PubMed: 22547573]
- [31]. Motokawa T, Kato T, Hashimoto Y, Katagiri T. Effect of Val92-Met and Arg163Gln variants of the MC1R gene on freckles and solar lentigines in Japanese. *Pigment Cell Res* 2007;20(2):140–3. [PubMed: 17371441]
- [32]. Filitis DC, Mahalingam M. Cutaneous adverse events to type I BRAF inhibitors: an analysis of effects associated with each inhibitor and therapeutic time interval to onset. *Am J Clin Dermatol* 2013; 14(6):461–71. [PubMed: 24048637]

Research in context

Evidence before this study

According to the current knowledge, patients who survive melanoma are at increased risk of developing keratinocyte skin cancer (KSC). Population- and hospital-based studies have demonstrated this fact. Although many environmental and clinical factors increase the risk for developing both melanoma and KSC, no prospective study to date has assessed in detail if these risk factors also increase KSC risk in melanoma survivors.

Added value of this study

In this study, we have shown cumulative incidence figures of non-melanoma skin cancer and related risk factors in a large cohort of melanoma patients. The estimated cumulative incidence exceeds by far that of the general population. Besides the obvious effect of advanced age, male sex, severe sunburns, previous diagnosis of keratinocyte skin cancer (KSC), chronic sun exposures and the presence of two polymorphisms in melanocortin 1 receptor (*MC1R*) (p.D294H and p.R163Q) were the most relevant risk factors for developing subsequent KSC.

Implications of all the available evidence

Our results indicate that some characteristics, including some melanocortin 1 receptor (*MC1R*) variants, are helpful to define melanoma patients at higher risk of developing keratinocyte skin cancer and therefore might benefit of implemented preventive measures.

Fig. 1.

Receiver-operating curves for combined outcome (keratinocyte skin cancer [KSC]) models. The black line corresponds to a model that predicts 5-year KSC risk based on age, sex, past history of sunburns, melanoma site, smoking, lentigo maligna melanoma subtype, actinic keratosis, red hair colour, and eye colour. The red line corresponds to a model that added p.R163Q to the clinical variables. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Fig. 2. Five-year predictions from a keratinocyte skin cancer cumulative incidence model that includes age, sex, past history of sunburns, melanoma site, smoking, lentigo maligna melanoma subtype, actinic keratosis, red hair colour, eye colour, and p.R163Q.

Table 1.

Characteristics of the study population (N = 1200).

Characteristics	Median	Range
Age at first primary melanoma diagnosis	56.8	18.4–96.1
	N	%
Gender		
Female	608	50.67
Man	592	49.33
Stage of melanoma at diagnosis		
<i>In situ</i>	181	15.08
Stage I/II	837	69.75
Stage III	173	14.42
Stage IV	7	0.58
NOS	2	0.17
Phototype		
I–II	428	35.7
III–V	734	61.2
Missing	38	3.2
Hair colour		
Black/brown	876	73.0
Blond	234	19.5
Red	52	4.3
Missing	38	3.2
Eye colour		
Dark (black/brown)	713	59.4
Fair (blue/green)	455	37.9
Missing	32	2.7
Lifetime severe sunburns		
5	925	77.1
>5	215	17.92
Missing	60	5

Characteristics	Median	Range
Chronic sun exposure		
No	804	67.0
20 years	100	8.3
>20 years	168	14.0
Missing	128	10.67
Smoking (pack-years)		
Non-smoker	580	48.3
20	280	23.3
>20	234	19.5
Missing	106	8.8
Presence of solar lentigines		
No	125	10.4
Yes	985	82.1
Missing	90	7.5
Personal history of other neoplasias		
No	1063	88.5
Yes	136	11.3
Missing	1	0.1
AK		
No	905	75.4
Yes	153	12.8
Missing	142	11.8
Number of common nevi		
<20	732	61.0
20–50	156	13.0
51–100	109	9.1
>100	52	4.3
Missing	151	12.6
Family history of melanoma		
No	1095	91.3
Yes	74	6.2

Characteristics	Median	Range
Missing	31	2.6
Family history of other non-cutaneous neoplasias		
No	660	55.0
Yes	491	40.9
Missing	49	4.1
Melanoma site		
Head/neck	248	20.7
Upper extremities	169	14.1
Trunk	456	38.0
Lower extremities	224	18.2
Acral	103	8.6
Histological subtype		
Lentigo maligna/LMM	130	10.8
Superficial spreading	727	60.6
Nodular	221	18.4
Acral lentiginous	54	4.5
Others/unclassified	68	5.7
MCIR variants		
0	338	28.2
1	410	34.2
>1	237	19.8
Missing	215	17.9
MCIR 'R' variants		
No	647	53.9
Yes	311	25.9
Missing	242	20.2
Personal history of KSC		
No	1120	93.3
Yes	80	6.7
Personal history of BCC		
No	1131	94.3

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Characteristics	Median	Range
Personal history of SCC		
Yes	69	5.8
No	1181	98.4
Yes	19	1.6

AK, actinic keratosis; BCC, basal cell carcinoma; KSC, keratinocyte skin cancer; LMM, lentigo maligna melanoma; *MC/R*, melanocortin 1 receptor; SCC, squamous cell carcinoma; NOS, not otherwise specified.

Table 2.

Associations of risk factors with risk of BCC, SCC and KSC overall in melanoma survivors adjusted for age in categories.

Feature	Combined, HR (95% CI)	BCC, HR (95% CI)	SCC, HR (95% CI)
Age[*]			
Q1	Ref	Ref	Ref
Q2	2.27 (1.11–4.66)	2.5 (1.19–5.25)	1.06 (0.07–16.91)
Q3	7.13 (3.76–13.55)	7.54 (3.86–14.72)	6.83 (0.82–56.76)
Q4	9.68 (5.09–18.42)	9.51 (4.85–18.66)	18.84 (2.45–145.04)
Sex			
Male	Ref	Ref	Ref
Female	0.5 (0.36–0.69)	0.53 (0.38–0.74)	0.17 (0.05–0.57)
Phototype			
I–II	Ref	Ref	Ref
III–V	0.82 (0.60–1.14)	0.83 (0.60–1.15)	0.3 (0.16–0.94)
Hair colour			
Black/brown	Ref	Ref	Ref
Blonde	0.67 (0.43–1.04)	0.67 (0.43–1.05)	1.18 (0.38–3.68)
Red	2.37 (1.38–4.06)	2.11 (1.18–3.75)	6.12 (1.97–19.03)
Eye colour			
Dark	Ref	Ref	Ref
Fair	1.17 (0.85–1.60)	1.09 (0.78–1.51)	4.72 (1.71–13.00)
Lifetime severe sunburns			
5	Ref	Ref	Ref
>5	2.06 (1.44–2.95)	2.09 (1.45–3.02)	1.48 (0.49–4.46)
Chronic sun exposure			
No	Ref	Ref	Ref
20 years	0.64 (0.32–1.26)	0.67 (0.34–1.33)	0.67 (0.09–5.15)
>20 years	0.85 (0.56–1.29)	0.82 (0.53–1.28)	0.98 (0.34–2.87)
Smoking			
No	Ref	Ref	Ref
20 pack-years	1.34 (0.85–2.12)	1.12 (0.69–1.83)	4.84 (1.66–14.13)

Feature	Combined, HR (95% CI)	BCC, HR (95% CI)	SCC, HR (95% CI)
>20 pack-years	1.56 (1.08–2.23)	1.48 (1.02–2.14)	2.47 (0.82–7.38)
Presence of solar lentigines			
No	Ref	Ref	Ref
Yes	1.49 (0.78–2.84)	1.38 (0.73–2.64)	–
Actinic keratoses			
No	Ref	Ref	Ref
Yes	2.25 (1.55–3.25)	2.09 (1.43–3.07)	5.52 (1.95–15.65)
Personal history of KSC			
No	Ref	Ref	Ref
Yes	3.65 (2.51–5.3)	3.61 (2.47–5.31)	5.59 (2.27–13.76)
Personal history of BCC			
No	Ref	Ref	Ref
Yes	3.85 (2.61–5.66)	3.74 (2.51–5.57)	6.74 (2.74–16.58)
Personal history of SCC			
No	Ref	Ref	Ref
Yes	2.44 (1.19–5.01)	2.24 (1.04–4.83)	6.15 (1.78–21.25)
Personal history of other non-cutaneous malignancy			
No	Ref	Ref	Ref
Yes	1.78 (1.23–2.59)	1.80 (1.23–2.65)	1.99 (0.77–5.18)
Family history of melanoma			
No	Ref	Ref	Ref
Yes	0.86 (0.40–1.85)	0.63 (0.26–1.54)	2.36 (0.54–10.36)
Family history of non-cutaneous malignancy			
No	Ref	Ref	Ref
Yes	1.03 (0.75–1.41)	1.07 (0.77–1.49)	1.75 (0.68–4.52)
Common melanocytic nevus			
<20	Ref	Ref	–
20–50	1.31 (0.80–2.15)	1.41 (0.86–2.30)	–
50–100	1.32 (0.67–2.59)	1.41 (0.72–2.78)	–
>100	1.21 (0.48–3.01)	1.29 (0.52–3.32)	–
Melanoma site			

Feature	Combined, HR (95% CI)	BCC, HR (95% CI)	SCC, HR (95% CI)
Head/neck	Ref	Ref	Ref
Upper extremities	0.99 (0.57–1.73)	1 (0.56–1.76)	0.39 (0.05–3.27)
Trunk	1.42 (0.94–2.16)	1.42 (0.92–2.19)	1.28 (0.43–3.80)
Lower extremities	1.24 (0.75–2.03)	1.25 (0.75–2.09)	1.03 (0.25–4.26)
Acral	0.65 (0.32–1.30)	0.71 (0.35–1.43)	1.3 (0.32–5.21)
Histological subtype			
LMM	Ref	Ref	Ref
Non-LMM	1.06 (0.07–16.9)	0.67 (0.44–1.02)	0.67 (0.24–1.86)
MC1R variants			
p.R151C			
WT	Ref	Ref	Ref
Het	1.19 (0.71–2.02)	1.09 (0.62–1.90)	2.15 (0.61–7.61)
Hom	6.96 (0.93–51.74)	7.19 (0.96–53.56)	–
p.R142H			
WT	Ref	Ref	Ref
Het	0.93 (0.38–2.29)	0.99 (0.41–2.44)	1.51 (0.20–11.40)
Hom	–	–	–
p.R160W			
WT	Ref	Ref	–
Het	1.49 (0.80–2.77)	1.58 (0.85–2.94)	–
Hom	–	–	–
p.D294H			
WT	Ref	Ref	Ref
Het	1.84 (1.13–2.99)	1.68 (1.00–2.80)	3.32 (1.07–10.33)
Hom	9.46 (3.44–25.97)	7.13 (2.24–22.70)	19.14 (2.36–155.17)
p.R163Q			
WT	Ref	Ref	Ref
Het	2.03 (1.09–3.78)	2.23 (1.20–4.13)	1.75 (0.23–13.48)
Hom	–	–	–

BCC, basal cell carcinoma; CI, confidence interval; Het, heterozygous; hom, homozygous; HR, hazard ratio; KSC, keratinocyte skin cancer; LMM, lentigo maligna melanoma; MC1R, melanocortin 1 receptor; Ref, reference; SCC, squamous cell carcinoma; WT, wild type.

* Age grouped into quartiles: Q1: <43.7; Q2: 43.7–56.8; Q3: 56.9–60.4; Q4: >60.4.

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Table 3.

Associations of risk factors with risk of BCC, SCC and overall NMSC from multivariable Cox regression models in melanoma survivors.

Characteristics	NMSC (BCC and SCC combined), HR (95% CI)	BCC, HR (95% CI)	SCC, HR (95% CI)
Age *			
Age in categories	2.07 (1.68–2.55)	2.02 (1.64–2.49)	2.81 (1.28–6.19)
Sex			
Male	Ref	Ref	–
Female	0.45 (0.31–0.67)	0.45 (0.30–0.66)	
Hair colour			
Black/brown	–	–	Ref
Blonde			0.39 (0.10–1.51)
Red			2.74 (0.73–10.30)
Eye colour			
Dark	–	–	Ref
Fair			5.94 (1.81–19.43)
Lifetime severe sunburns			
5	Ref.	Ref.	–
>5	1.55 (1.03–2.33)	1.47 (0.97–2.23)	
Chronic sun exposure			
No	Ref	Ref	–
20 years	0.40 (0.18–0.86)	0.40 (0.19–0.88)	
>20 years	0.46 (0.27–0.78)	0.42 (0.24–0.72)	
AK			
No	–	–	Ref
Yes			4.44 (1.31–15.01)
Personal history of KSC			
No	Ref	Ref	–
Yes	2.43 (1.54–3.83)	2.8 (1.77–4.43)	
Personal history of other non-cutaneous malignancy			
No	Ref	Ref	–
Yes	1.56 (1.02–2.41)	1.52 (0.97–2.37)	

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Characteristics	NMSC (BCC and SCC combined), HR (95% CI)	BCC, HR (95% CI)	SCC, HR (95% CI)
RHC variants			
No	–	Ref	–
I		1.53 (1.05–2.24)	
p.D294H			
WT	Ref	–	–
Mut	1.92 (1.19–3.09)		
p.R163Q			
WT	Ref	Ref	–
Mut	2.09 (1.12–3.92)	2.19 (1.17–4.10)	

AK, actinic keratosis; BCC, basal cell carcinoma; CI, confidence interval; HR, hazard ratio; KSC, keratinocyte skin cancer; LMM, lentigo maligna melanoma; mut. mutated; Ref, reference; RHC, red hair colour; SCC, squamous cell carcinoma; WT, wild type.

Only variables that were significant at $p < 0.05$ were retained in the models.

* Age cutoffs (quartiles): Q1: <43.7; Q2: 43.7–56.8; Q3: 56.9–60.4; Q4: >60.4; not significant.