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Meta-analysis of risk factors for cutaneous melanoma according to anatomical site and clinico-pathological variant

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ABSTRACT

A systematic meta-analysis was performed to evaluate if cutaneous melanoma (CM) risk factors differ depending on body site and histological type. Adjusted estimates were extracted from 24 observational studies, for a total of 16,180 cases. Multivariate random-effects models were used to obtain summary relative risk (RR) estimates for all risk factors by body site and histological type. Summary RRs suggest that high naevus counts are strongly associated with CM on usually not sun exposed sites ($p < 0.001$) while different patterns of sun exposure show a tendency for higher RRs for CM on usually sun exposed sites than on other body sites ($p = 0.087$). Continuous pattern was found to be significantly inversely associated with CM for unexposed sites ($p = 0.01$). RRs also differed by body site for skin ($p = 0.01$) and hair colour ($p = 0.01$), and these differences could be attributed to gene variability. This finding seems to suggest different aetiologic pathways of melanoma development by anatomical site.

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1. Introduction

Starting from the 1960s, incidence rate of cutaneous melanoma (CM) has steadily increased in Caucasian populations worldwide, in both men and women and in all age groups. Despite a recent flattening of this trend, CM has become one of the most frequent cancers in fair-skinned populations.¹ In a similar way, the increase in mortality rates involving both sexes and most age groups has been followed since the mid-1990s by a slowdown or even a reversal of the trend.² The high-

est incidence and mortality rates are observed in Australia, Northern Europe and South Africa. Age-standardised incidence rates tend to be higher in females in European countries and in males elsewhere; mortality is generally higher in men.¹

As for all cancers, the occurrence of CM is the result of the interaction between host and environmental factors. While the main constitutional and environmental risk factors for CM are well known,³ it remains unclear how these risk factors interact to determine the anatomical site and histological type of the developing tumour.

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Based on the existing epidemiological data and clinical observations, Green⁴ proposed a theory of site-dependent susceptibility of melanocytes to malignant transformation. According to this hypothesis, people with a low propensity for melanocyte proliferation need a continuous exposure to sunlight in order to drive the clonal expansion of initiated melanocytes. Melanomas developing in this pathway are more likely to be located on sun exposed body sites, to be of lentigo maligna melanoma (LMM) subtype and to occur in older patients with a history of solar damage and non-melanocytic skin cancer (NMSC). On the contrary, individuals with a high propensity for melanocyte proliferation, as indicated by a large number of common naevi, tend to develop melanomas on intermittently sun exposed body sites, belonging to the superficial spreading melanoma (SSM) or nodular melanoma (NM) histological subtypes and showing little if any association with a history of NMSC or sun-induced skin damage.

We recently published a series of three meta-analyses^{5–7} that quantified the increase in risk of CM associated with continuous and intermittent sun exposure, sunburns, indicators of actinic damage, skin, eye and hair colour, skin type and freckles. Overall, the picture outlined by the results of these works left ample room for discussion, above all due to the unexplained, divergent effects of intermittent and continuous sun exposure: the present meta-analysis aims therefore to complete and refine the results of our previous works, by performing a further meta-analysis in which CM has been separately analysed with regard to histological type and anatomical site.

2. Patients and methods

2.1. Definition of the outcomes and exposure

The outcome variables in this systematic meta-analysis are histologically confirmed CMs on particular anatomical sites or of particular pathological types. Mucosal melanomas and CMs situated on palms, soles, fingers and toes were excluded from the analysis. We also restricted our analysis to the most common histopathological types, LMM, SSM and NM.

Detailed definitions of sun exposure and phenotype variables are given in our previous reports.⁵ Briefly, we investigated the following risk factors: continuous and intermittent sun exposure, sunburns, actinic damage indicators (actinic keratoses, NMSC, solar lentigo and elastosis), skin, eye and hair colour, skin type, freckles and common melanocytic naevi. Family history of CM and clinically atypical naevi were excluded due to the small numbers of site- and type-specific estimates of their effects.

2.2. Data sources and search strategy

For the implementation of our previous meta-analyses⁵ we searched all the publications listed in MEDLINE and EMBASE databases up to 30th September 2002, according to the following inclusion criteria: only human observational studies; a case-control, cohort, cross-sectional or case-only design; and providing sufficient information to estimate a measure of relative risk (RR) and its 95% confidence interval (CI) for the association between an exposure of interest and CM.

Starting from this initial pool of papers (encompassing also a few papers not included in our previous analyses), we extended the search for new articles to those published up to 31st July 2007 and checked in the reference list of papers collected up to that point for other articles that met the criteria for inclusion. We finally selected the papers where analyses of one or more risk factors for CM were done according to anatomical site or histological type.

From each study, the following information was collected: general information (study design, year of publication and study country), definitions of exposure and outcome, statistical methods used for the analysis and variables taken into account as potential confounders, main results (number of cases and controls, in the aggregate and broken down by body site and histological type, and effect estimates with their 95% CI) and data necessary for their correct interpretation (source and mean age of cases and controls).

When two or more articles were based on the same study sample, we used the estimate based on the largest population or, if the size was the same, that most adjusted for relevant confounding variables.

2.3. Methods of analysis

The distinction among the various measures of RR (e.g. odds ratio, rate ratio and risk ratio) was ignored on the assumption that melanoma is a rare disease. Consequently, for any different pairs of exposure and outcome subgroups the most adjusted measures of association and the corresponding CI were translated into log (RR) and the corresponding variance with the formula proposed by Greenland.⁸ When estimates were not available from the paper, they were calculated from the published crude data. If only the *p*-value was published, we obtained a 'test-based' estimate.⁸

For patterns of sun exposure, sunburns and freckles, we used the RR estimates for the highest level in order to reduce the possibility of exposure misclassification.

The summary RR for each exposure and outcome (melanoma on a particular body site or of a specific histological type) was obtained from a multivariate mixed effects models with maximum likelihood estimates using PROC MIXED in SAS (SAS Institute Inc. SAS Windows version (8.02), 1999, Cary, NC) and by taking into account between-study variability and correlations of all estimates coming from the same study.⁹ RRs for body sites were estimated for each category in the following three categorisations of sites: individual broad sites (head and neck, arms, trunk and legs); the site categories 'trunk' and 'non-trunk'; and 'usually sun exposed body sites' (arms and head or sites classified as 'sun exposed' by the authors) and 'occasionally exposed body sites' (lower limbs and trunk or sites classified as 'not sun exposed' by the authors).

Summary estimates of the risk for an increase in the number of naevi were based on a two-step procedure. In the first step, a linear model was fitted, within each study, to estimate the relative risk for an increase of one naevus. When sufficient information was published (the number of subjects in each category of naevi), the model was fitted according to the method proposed by Greenland and Longnecker,¹⁰ which provides the natural logarithm of RR and an estimator of its

standard error by taking into account that the estimates for separate categories depend on the same reference group. When the number of subjects in each category was not available from the papers, coefficients were calculated by ignoring the correlation between the estimates of risk in the separate exposure levels. In the second step, the summary RR was estimated by pooling the study-specific estimates by the use of random-effects models.⁸

Heterogeneity across studies was evaluated by the Q statistic (Chi-square test) and by I^2 , which represents the percentage of total variation across studies that is attributable to heterogeneity rather than to chance.¹¹ We considered that statistically significant heterogeneity existed when the *p* value from the Chi-square test was less than 0.10.

To evaluate the differences in the effects of risk factors by body sites and histology, analyses were carried out using a multivariate approach proposed by van Houwelingen and colleagues⁹ on the outcomes analysed together. Once the model was fitted, the differences on effect estimates by body site or histology, modelled as fixed parameter, were tested with Wald test.

Heterogeneity analysis was carried out, using the same multivariate approach, by including in the meta-regression factors that could affect the results (e.g. country of the study, source of controls and year of publication). Then, for each level of meta-regression factor, RRs and 95% CIs were obtained with least squares means procedures. For this analysis risk factors were grouped (RRs related to sun exposure and RRs for phenotypic factors) in order to have more power and to carry out subgroup analyses with greater numbers of studies.

The hypothesis that publication bias might affect the validity of the estimates was tested by funnel-plot-based approaches using the adjusted rank correlation method (Begg's method¹²) and linear regression analysis on a radial plot (Egger's method¹³). Risk factors for which publication bias was suggested by the results of Egger's and Begg's tests were further investigated by using the Copas and Shi method^{14,15} to determine how the summary RR would change adjusted for publication bias. Sensitivity analysis was carried out to evaluate whether the results could have been influenced by the inclusion criteria (e.g. the exclusion of case–case studies). The influence of single papers was also assessed.

To correct for multiple comparisons we calculated the *q* values proposed by Storey and Tibshirani.¹⁶ The *q* values are similar to the well-known *p* values, except that they give the expected proportion of false positive incurred when calling an estimate significant. The *q* values were calculated using the R library *q*-value.

3. Results

We identified 36 published articles with RRs for associations of sun exposure and phenotypic characteristics with CM by body site or histological type. Four papers were excluded because they were case–case studies, comparing risks by body site with the risk on the trunk,^{17–20} and three were excluded because the authors analysed only acral melanomas.^{21–23}

The present meta-analysis includes a total of 16,180 cases of CMs from 29 published papers and 24 independent study

groups (Table 1). The first study was published in 1984 and the last one in 2006. Twelve studies were carried out in the European countries, nine in the United States or Canada and three in Australia. All studies used the case–control design, except for four cohort studies.

Forrest plots for each exposure considered and outcome (melanoma on a particular body site or of a specific histological type) are presented in the Appendix.

Summary RRs for intermittent sun exposure, continuous sun exposure and sunburns were each higher on usually sun exposed sites than on occasionally sun exposed sites and this difference was statistically significant for continuous sun exposure (*p* = 0.01; Table 2). Overall, however, there was a negative association of continuous pattern of sun exposure with CM, which was confined to CM on occasionally sun exposed sites. The effects of the three measures of sun exposure did not vary in any consistent way by the subtype of melanoma.

Like the direct measures of sun exposure, the presence of actinic damage indicators was more strongly associated with CM on usually sun exposed body sites, although the difference was small. Their association with CM did not vary significantly among the three subtypes (*p* = 0.83). Skin colour and skin type were positively associated with CM on both usually and occasionally sun exposed body sites but the association of skin colour with CM was significantly stronger on usually exposed body sites (*p* = 0.01). Both skin colour and skin type were positively associated with each histological subtype.

Freckling, hair colour and eye colour were positively and significantly associated with CM on all body sites. The association of hair colour with CM, however, was significantly stronger on occasionally sun exposed body sites (*p* = 0.01). Each of these three phenotypic factors was significantly associated with SSM and NM but showed little evidence of an association with LMM. Hair colour was more strongly associated with SSM than with the other histological types (*p* < 0.05).

Presentation of the summary RRs according to an anatomical classification of the skin (Table 3) generally showed a similar picture of the sites' patterns of sun exposure, even if statistical significance was not always achieved due to small numbers. The negative association of continuous sun exposure was stronger for CM on the legs than on the trunk; the stronger associations of sunburns and skin colour with CM on usually exposed sites are reflected in the stronger associations with CM on the head and arms. The stronger association of hair colour with CM on occasionally exposed sites is similarly reflected in the stronger associations with CM on trunk and legs.

The sensitivity analysis we performed on the results given in Table 3 by including also case–case studies showed no appreciable changes in the summary RRs except for chronic sun exposure, where with the inclusion of two case–case studies the estimates for head and neck melanoma rose to 1.56 (95% CI: 0.90–2.70).

The strongest association with CM in general (RR = 1.79; 95% CI: 1.56–2.06) was seen for the increase of five naevi on the legs (Table 4), while the weakest association was seen for naevi on the head (RR = 1.42; 95% CI: 1.23–1.64) (*p* < 0.001 for difference among RRs). Concerning the association between naevi in general and site-specific CM, the strongest

Table 1 – Published studies reporting Cutaneous Melanoma (CM) estimates for several risk factors, by histological type, anatomical site or both.

First author	Country	Year	Study design	Controls source	No. of cases	No. of controls	Intermittent sun exposure	Continuous sun exposure	Sun-burns	Actinic damage indicators	Skin colour	Skin type	Freckles	Hair colour	Eye colour	Common naevi
Holman ^{24,b,c}	Australia	1984	CC	Pop	511	511	x	x	x	x		x				x
Green ^{27,a}	Australia	1986	CC	Pop	183	183			x							
Swerdlow ^{28,a,b}	Scotland	1986	CC	Hosp	180	197										x
Vagero ^{29,a}	Sweden	1986	Cohort	–	4706	–		x								
Cristofolini ^{30a}	Italy	1987	CC	Hosp	103	205	x	x			x			x		
Elwood ^{31,b}	Canada	1987	CC	Pop	599	599	x	x	x		x	x	x	x		
Østerlind ^{32,a,b}	Denmark	1988	CC	Pop	474	926					x	x	x	x		x
Weinstock ^{33,a}	USA	1989	NCC	Pop	110	231										x
Weiss ^{35,b}	Germany	1991	CC	Hosp	204	200	x	x				x		x		x
Stierner ^{34,a}	Sweden	1992	CC	Hosp	121	310										x
Krüger ^{36,a}	Germany	1992	CC	Hosp	200	200										x
Herzfeld ^{37,a}	USA	1993	CC	Pop	324	415	x	x			x	x	x	x	x	
Goodman ^{38,a}	USA	1995	CC	Other	3527	53,129		x								
Cress ^{39,a,b,d}	USA	1995	CC	Pop	452	930	x	x	x		x	x	x	x	x	
Rieger ^{42,a}	Germany, Austria	1995	CC	Hosp	278	278										x
Chen ^{43,a}	USA	1996	CC	Pop	548	494	x	x	x		x	x	x	x	x	x
Grulich ^{44,a}	Australia	1996	CC	Hosp	244	276										x
Ródenas ^{45,b}	Spain	1997	CC	Other	116	116										x
Masback ^{46,a,b}	Sweden	1999	CC	Pop	366	640		x	x				x			x
Walter ^{47,a,b}	Canada	1999	CC	Pop	583	608	x	x	x							
Langholz ^{48b}	USA	2000	CC	Pop	773	752					x	x	x	x	x	x
Håkansson ^{49,a}	Sweden	2001	Cohort	–	525	–		x								
Cho ^{50,a}	USA	2005	Cohort	–	511	–			x					x		
Naldi ^{51,a,b,e}	Italy	2005	CC	Hosp	542	538			x	x	x	x	x	x	x	x

x, Relative risk estimates published in the articles for the indicated risk factor. NCC: nested case–control study. CC: case–control study. Hosp: hospital-based study. Pop: population-based study. Other: other types of controls.

a Articles that presented estimates by body sites.

b Articles that presented estimates by histology.

c Data extracted also from Holman^{25a} and Holman.^{26b}

d Data extracted also from Holly⁴⁰ and Holly.^{41b}

e Data extracted also from Randi.^{52a}

Table 2 – Summary relative risks (RRs) of cutaneous melanoma with 95% confidence intervals (CIs) for sun exposure and phenotypic characteristics by body site, classified according to sun exposure pattern, and histological type.

	n ^e	RR (95% CI)	p Value ^a	I ²	n ^e	RR (95% CI)	p Value ^a	I ²	n ^e	RR (95% CI)	p Value ^a	I ²
	Intermittent sun exposure High versus low				Continuous sun exposure High versus low				Sunburns Many versus few			
All	23	1.28 (1.07–1.54)		67.4	35	0.91 (0.81–1.01)		49.3	48	1.83 (1.59–2.12)		54.6
Usually sun exposed ^c	3	2.03 (1.29–3.20)		0.0	8	1.09 (0.96–1.24)		43.7	13	2.33 (1.50–3.62)		43.3
Occasionally sun exposed ^d	5	1.16 (0.49–2.75)	0.17	89.9	9	0.90 (0.84–0.96)	0.01	75.1	15	1.71 (1.30–2.25)	0.06	60.8
LMM	4	1.07 (0.83–1.38)		0.0	4	1.07 (0.44–2.58)		0.0	4	1.58 (0.79–3.15)		0.0
NM	5	1.29 (0.82–2.05)		59.1	6	0.90 (0.71–1.13)		65.3	7	0.91 (0.50–1.65)		59.2
SSM	5	1.17 (0.96–1.43)	0.82	0.0	6	1.02 (0.71–1.47)	0.85	35.2	7	1.56 (1.26–1.93)	0.25	30.4
	Actinic damage indicators Some versus none				Skin colour Fair versus dark				Skin type Burns easily versus always tans			
All	13	1.56 (1.28–1.91)		24.9	31	1.86 (1.67–2.06)		0.0	36	1.67 (1.39–2.01)		54.8
Usually sun exposed ^c	2	1.54 (1.02–2.31)		0.0	9	2.78 (2.09–3.70)		0.0	8	1.67 (0.82–3.41)		77.1
Occasionally sun exposed ^d	2	1.40 (1.07–1.82)	0.70	0.0	10	1.78 (1.50–2.12)	0.01	0.0	9	1.70 (1.29–2.24)	0.97	0.0
LMM	3	2.36 (0.82–6.82)		66.3	2	2.33 (1.14–4.77)		0.0	5	2.25 (0.86–5.90)		63.9
NM	3	1.88 (0.63–5.61)		17.5	5	1.91 (1.37–2.66)		0.0	7	1.89 (1.11–3.22)		29.7
SSM	3	1.94 (1.13–3.34)	0.83	52.9	5	1.60 (1.33–1.92)	0.44	29.9	7	1.40 (1.06–1.86)	0.65	66.8
	Freckles Many versus few				Hair colour Light versus dark				Eye colour Light versus dark			
All	23	1.79 (1.60–2.00)		0.0	90	1.52 (1.40–1.66)		55.9	31	1.70 (1.54–1.87)		0.0
Usually sun exposed ^c	5	1.83 (1.23–2.74)		0.0	26	1.46 (1.22–1.73)		37.0	8	2.04 (1.59–2.61)		0.0
Occasionally sun exposed ^d	6	1.96 (1.58–2.43)	0.77	0.0	28	1.73 (1.49–2.01)	0.01	51.7	9	1.55 (1.32–1.82)	0.07	0.0
LMM	1	0.80 (0.28–2.31)		–	6	1.01 (0.84–1.22)		0.0	2	0.90 (0.46–1.78)		0.0
NM	5	1.71 (1.23–2.37)		0.0	15	1.32 (1.08–1.62)		57.0	6	1.95 (1.37–2.77)		0.0
SSM	5	1.83 (1.55–2.17)	0.31	9.2	15	1.61 (1.35–1.93)	<0.05 ^b	55.8	6	1.72 (1.42–2.09)	0.13	10.5

a p Values for differences among categories. LMM: Lentigo maligna melanoma. NM: Nodular melanoma. SSS: Superficial spreading melanoma. I² percentage of total variation attributable to heterogeneity rather than to chance.

b Mixed models did not converge and we could not get precise p values.

c 'Arms', 'head' and 'sun exposed'.

d 'Trunk', 'lower limbs' and 'not sun exposed'.

e Number of estimates used to calculate the RR for each association.

Table 3 – Summary relative risks (RRs) of cutaneous melanoma with 95% confidence intervals (CIs) for sun exposure and phenotypic characteristics by body site classified anatomically.

	<i>n</i> ^d	RR (95% CI)	<i>p</i> Value ^a	<i>n</i> ^d	RR (95% CI)	<i>p</i> Value ^a	<i>n</i> ^d	RR 95% CI	<i>p</i> Value	<i>n</i> ^d	RR (95% CI)	<i>p</i> Value ^a
	Intermittent sun exposure High versus low			Continuous sun exposure High versus low			Sunburns Many versus few			Skin colour Light versus dark		
Trunk	3	1.82 (1.18–2.80)		5	0.91 (0.73–1.13)		9	1.81 (1.28–2.59)		5	1.71 (1.37–2.15)	
Head	1	2.60 (1.20–5.62)		4	0.99 (0.66–1.47)		7	2.43 (1.54–3.83)		4	3.21 (1.89–5.45)	
Arms	1	2.40 (1.20–4.80)		2	0.75 (0.48–1.19)		6	2.26 (1.27–4.02)		4	2.78 (1.91–4.03)	
Legs	1	2.70 (1.23–5.94)		2	0.44 (0.21–0.91)	0.16	6	1.66 (1.23–2.23)	0.33	4	1.76 (1.31–2.35)	0.03
Not trunk ^c	4	1.59 (0.90–2.80)	0.48	10	0.76 (0.58–0.99)	0.15	21	1.99 (1.57–2.52)	0.46	12	2.22 (1.78–2.77)	0.09
	Skin type Burns easily versus always tans			Freckles Many versus few			Hair colour Light versus dark			Eye colour Light versus dark		
Trunk	5	1.80 (1.32–2.45)		4	1.94 (1.54–2.45)		14	1.77 (1.52–2.06)		5	1.57 (1.30–1.91)	
Head	4	1.59 (0.56–4.49)		3	1.65 (0.97–2.82)		13	1.42 (1.08–1.89)		4	2.16 (1.53–3.03)	
Arms	4	1.63 (0.78–3.43)		2	2.10 (1.14–3.85)		12	1.51 (1.17–1.94)		4	1.91 (1.33–2.75)	
Legs	4	1.59 (0.99–2.55)	0.98	2	2.09 (1.15–3.79)	0.93	13	1.67 (1.29–2.17)	<0.05 ^b	4	1.51 (1.14–1.99)	0.31
Not trunk ^c	12	1.61 (0.89–2.91)	0.73	8	1.65 (1.27–2.14)	0.36	38	1.54 (1.33–1.79)	<0.01	12	1.78 (1.48–2.14)	0.36

a *p* Values for differences among categories.

b Mixed models did not converge and we could not get precise *p* values.

c *p* Value for the difference between ‘Trunk’ and ‘Not trunk’ (‘arms’, ‘extremities’, ‘head’, ‘limbs’ and ‘not trunk’).

d Number of estimates used to calculate the OR for each association.

Table 4 – Summary relative risks (RRs) and 95% confidence intervals (CIs) of cutaneous melanoma at different body sites, due to the increase of five common naevi at different body sites.

	Site of melanoma					<i>I</i> ²
	Arms	Head	Legs	Trunk	Whole body	
Naevi site						
Arms	1.40 (1.20–1.62)	1.53 (1.27–1.84)	1.58 (1.36–1.82)	1.66 (1.44–1.92)	1.60 (1.39–1.83)	58.3
Head	0.99 (0.81–1.20)	1.51 (1.25–1.83)	1.45 (1.22–1.71)	1.50 (1.26–1.78)	1.42 (1.23–1.64)	81.9
Legs	1.59 (1.35–1.87)	1.44 (1.20–1.71)	1.89 (1.63–2.18)	1.86 (1.59–2.16)	1.79 (1.56–2.06)	87.5
Trunk	1.46 (1.22–1.73)	1.52 (1.26–1.83)	1.54 (1.31–1.80)	1.88 (1.62–2.18)	1.67 (1.45–1.92)	75.5
Whole body	1.34 (1.16–1.54)	1.50 (1.30–1.73)	1.60 (1.40–1.84)	1.72 (1.50–1.97)		
<i>I</i> ^{2a}	74.5	76.2	77.8	75.7		

a *I*² percentage of total variation attributable to heterogeneity rather than to chance.

association was seen for CM on the trunk (RR = 1.72; 95% CI: 1.50–1.97) and the lowest association was seen for CM on the arms (RR = 1.34; 95% CI: 1.16–1.54) ($p < 0.001$). The number of naevi on the trunk and the number of naevi on the legs were each more strongly associated with melanoma on the same site than on other sites. There was significant heterogeneity in the matrix of RRs by site of naevi and site of melanoma ($p = 0.005$).

Finally, naevi count resulted positively associated with melanoma of each histological type, without heterogeneity among RRs: 1.22 (95% CI: 1.08–1.37), 1.31 (95% CI: 1.18–1.45) and 1.32 (95% CI: 1.21–1.44), for LMM, NM and SSM, respectively.

A correction for multiple comparisons was made through the false discovery rate methodology. In most cases, significant associations were confirmed after correction; q values for associations between hair and skin colour and anatomical site, and between hair colour and histological type were no longer significant, but still lower than 0.10.

Investigation of between-study heterogeneity indicated that some characteristics explained variability among the estimates. The RRs for indicators of sun exposure (intermittent, chronic and sunburns) adjusting for the type of exposure, were significantly greater in cohort studies (2.47, 95% CI: 1.72–3.56) than in case-control studies (1.38, 95% CI: 1.11–1.71). When adjusted for phenotypic factors, the RR for continuous exposure was 0.51 (95% CI: 0.31–0.83), while when unadjusted it was 1.61 (95% CI: 1.01, 1.92; p for difference = 0.06). RRs for skin, hair and eye colour analysed together were lower in the European studies (RR = 1.48 with 95% CI: 1.19–1.83) than in the extra-European studies (RR = 1.95 with 95% CI: 1.61–2.35; p for difference = 0.02). In order to take into account the quality of diagnostic ascertainment, we classified the articles according to the presence or absence of a scheduled review by a panel of pathologists. This variable was found to explain part of the variability among estimates for chronic exposure and hair colour, but the differences among RRs remained nonetheless significant even after the introduction of this variable in the model.

Both Begg's and Egger's method found evidence of publication bias for analyses of hair colour ($p = 0.005$ and <0.001 , respectively), actinic damage indicators ($p = 0.024$ and 0.006) and number of naevi by both body site of naevi ($p = 0.002$ for each test) and CM ($p = 0.009$ and <0.001). Adding unpublished estimates in order to make publication bias unlikely, the sum-

mary RRs for hair colour RR = 1.17 (95% CI: 1.06–1.29), actinic damage indicators RR = 1.29 (95% CI: 1.07–1.57), site-specific naevi count RR = 1.49 (95% CI: 1.40–1.59) and site-specific CM RR = 1.46 (95% CI: 1.36–1.57) still remained significant.

We found that a few estimates were strongly sensitive to the exclusion of some papers from the analysis. Excluding Cristofolini et al.,³⁰ the RR for the association between intermittent sun exposure and CM on occasionally sun exposed skin sites increased to 1.93 (95% CI: 1.24–3.01). The exclusion of Ródenas et al.⁴⁵ and Swerdlow et al.²⁸ reduced the variation among estimates of the effects of site-specific naevus count on risk of CM as a whole and produced significantly higher RRs for the associations of total naevus count with NM and SSM compared to LMM.

4. Discussion

In this meta-analysis we have summarised the results of 24 separate studies of CM published before 31st July 2007, encompassing 16,180 cases of melanoma, and have focused on the results relating to the associations of sun exposure, pigmentary characteristics and melanocytic naevi with CM at particular body sites and of particular histological types. We included all applicable studies and used random-effects models taking into account variability within and between studies. We investigated heterogeneity by considering all possible sources of variability but could not explain in all cases the reasons for it. While we did not have individual original databases, which allow deeper investigation of sources of variability and adjustment for confounders in a consistent way, residual confounding should not have much effect on the results because we only included fully adjusted estimates.

We will discuss each set of associations separately and then summarise their joint implications for our understanding of melanoma aetiology.

4.1. Associations of sun exposure and pigmentary characteristics with CM at particular body sites

A higher RR for CM on usually sun exposed sites (or the head when an anatomical definition of body site was used) than on other body sites with each measure of sun exposure, including measures of actinic skin damage, was the most consistent pattern observed in these associations. If we consider each

measure of sun exposure (intermittent, chronic, sunburns and actinic damage) summary RRs, obtained taking into account the within study correlation, were 1.31 (95% CI: 0.94–1.81) and 1.77 (95% CI: 1.30–2.41), respectively, for occasionally and usually sun exposed body sites (p for difference = 0.087). Continuous sun exposure was weakly, but significantly, negatively associated with CM on occasionally sun exposed sites, most strongly on the legs. Pigmentary characteristics (skin colour, skin type, freckling, hair colour and eye colour) were positively associated with melanoma on each body site. These associations showed no consistent overall pattern of variation among the sites. There was though divergence in the patterns for skin type and hair colour, the RRs of which were, respectively, significantly higher for head and arms and usually exposed sites, and trunk and legs and occasionally exposed sites.

Our results are somewhat different from those of Chang and colleagues⁵³ in a pooled analysis of original data from 15 case-control studies of melanoma (four also included in our analysis). They showed generally higher ORs for sunbathing, recreational sun exposure and sunburn on the trunk than on the head and neck, though for a measure of total sun exposure and for the presence of solar keratoses on the face, the opposite was the case. There was also evidence that occupational sun exposure increased ORs for melanoma on the head and neck but not for melanoma of the trunk in populations living at low latitudes. Thus there may be heterogeneity in the site-specific relative risk of melanoma by the pattern of sun exposure that is not evident in our analysis. That overall risk of melanoma is generally higher on usually exposed body sites than on occasionally exposed body sites and is highest on the face in the European origin populations living across a range of ambient solar UV irradiances. This also consistent with a greater effect of overall sun exposure on usually exposed sites than on occasionally exposed sites.^{54,55}

Overall, our results suggest that sun exposure can cause CM to develop on any body site if it is exposed to the sun at all. This is best demonstrated by RRs for sunburns and actinic damage, which are significantly positive for any body site. The apparently protective effect of more continuous sun exposure against CM on occasionally exposed sites and, at most, weakly causal effect on usually exposed sites are puzzling. While enhanced melanin production and melanosome delivery to keratinocytes⁵⁶ and increased thickness of the top layers of the epidermis⁵⁷ due to the continuing sun exposure may offer a partial explanation, they would not be expected to reduce the incidence to a level below that present in the absence of sun exposure. It is important to note, however, that the reference category for calculating relative risks in epidemiological studies of melanoma and sun exposure is invariably 'low' sun exposure, not 'no' sun exposure. It is possible for analyses of continuous sun exposure that this category has generally contained higher proportions of people with high intermittent sun exposure than the higher continuous exposure categories. If that were so, an apparently protective effect of high continuous exposure might be observed, particularly perhaps for melanoma on intermittently exposed sites, as in this analysis. Unfortunately, a measure of total (lifetime) sun exposure was reported by only two studies included in our meta-analysis, so making impossible a more deep examination of this topic.

4.2. Associations between sun exposure and pigmentary characteristics and CM of particular histological types

Sun exposure measures and phenotypic characteristics were generally positively associated with each histological type of melanoma. NM, however, was not positively associated with sunburn, in contrast to LM and SSM, and LM was not positively associated with freckling, light eye colour and hair colour (RR = 1.01), in contrast to NM and SSM, which were significantly associated with all the three. These differences among histological types could have been due to chance, however, except possibly that with hair colour.

The distribution by body site of different histological types of CMs is uneven, with SSM being the more frequent type on the trunk in men and on the legs in women and LMM being found more frequently on the face and neck.⁵⁸ Thus, the patterns of associations of histological type of melanoma with sun exposure and phenotype may reflect those of body site of melanoma with these characteristics; the two are potentially quite highly confounded.

The lack of association of LM with hair colour, eye colour and freckles raises the possibility that it is less associated with variants in the MC1R gene than are NM and SSM; overall risk of CM is about twofold higher in carriers of red hair variants of this gene than in non-carriers.⁵⁹ This possibility is consistent with the evidence that MC1R variants are particularly associated with an increased risk of developing BRAF-positive CMs on skin not showing signs of chronic sun damage.⁶⁰ There is currently conflicting evidence on whether BRAF mutations are less frequent in LM than in other melanoma types.⁶¹ However, the only study, to our knowledge, that examined this hypothesis⁶² found a similar distribution of types of melanomas among MC1R carriers and non-carriers.

4.3. Associations of common melanocytic naevi overall and at particular body sites with CM as a whole, on particular body sites and of particular histological types

The associations of the number of naevi on specific body sites with the risk of melanoma on the same or other body sites are difficult to be summarised economically. Three features of them stand out however: total naevus count is more strongly associated with melanoma on the trunk and legs than on the head and arms; naevus counts on the legs and trunk are more strongly associated with melanoma in general than naevus counts on the head and arms; and the association of naevi on the head with melanoma on the arms was the only such association that was not significantly positive (RR = 0.99; 95%CI: 0.81–1.20). It appears, therefore, that naevi are more strongly associated with melanoma on the trunk and legs (occasionally sun exposed sites) than they are with melanoma on the head and arms (usually sun exposed sites). We found little evidence to suggest that the associations of the number of naevi with melanoma varied by histological type.

Our results are in good agreement with those of a recently published original pooled analysis of women in 10 case-control studies (five also included in this study) by Olsen and colleagues,⁶³ where positive associations with naevus count on the arm were found for CM situated on the trunk and upper

and lower limbs, while the association with CM on the head and neck, though positive, was much weaker (OR = 2.0; 95% CI: 0.9–4.5). It is possible, though, that information bias attenuates the association between the number of naevi and CM on sun exposed body sites, since CMs on these sites usually occur at an older age and the number of naevi decreases after the age of 30.⁶⁴

The greater association of naevus count with CM on intermittently sun exposed skin is consistent with the frequent detection of sporadic mutations on the BRAF gene in benign melanocytic lesions⁶⁵ and in CMs developing on the trunk and legs,⁶⁶ observations that support the dual pathway hypothesis advanced by Whiteman and colleagues.⁶⁷ Nonetheless, the observation that RRs are significantly elevated for almost any combination of sites of naevi and melanoma suggests that a large number of naevi is a risk factor for CM regardless of site.

5. Conclusion

Our results are broadly consistent with recent original pooled analyses in showing that pigmentary characteristics, a history of sunburns and a naevus-prone phenotype are risk factors for melanoma for the different body sites and histological types, even if with different magnitudes. The observed differences of RRs for naevi and patterns of sun exposure seem to confirm the existence of different aetiologic pathways of melanoma development by anatomical site. Moreover, with respect to skin, hair and eye colour and the LM histological type, differences in RRs point to the probable contribution of variation in the MC1R gene to melanoma's heterogeneity.

Conflict of interest statement

None declared.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ejca.2009.05.009](https://doi.org/10.1016/j.ejca.2009.05.009).

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