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The Effect of Sun Exposure in Determining Nevus Density in UK Adolescent Twins

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We report a study of 221 teenage twin pairs to examine the genetic and environmental determinants of nevi representing the most potent phenotypic risk factor for melanoma. Our published heritability analysis estimated that nevi are mainly genetically determined. In this paper we examine the role of sun exposure. We report a correlation between nevus density and sun exposure, particularly that acquired in hotter countries than in the UK (mean nevus density 41 per m^2 in those in the highest quartile of exposure *vs* 24 per m^2 in those with no exposure, $p < 0.0001$). We were not able to demonstrate a protective effect for either sun protection cream or shirt wearing. By including phenotypic variables and reported sun exposure into the heritability analysis, we conclude that 66% of the total variance of nevus count is attributable to genetic effects: 7% associated to eye color, 6% to hair color, and 1% to reported skin type, which leaves 52% as to yet unidentified genetic factors. Of the 25% of variation attributable to environmental influences, one-third is estimated to be because of sun exposure on hot holidays.

Key words: melanoma risk/nevi/sun exposure/sun protection/twin study
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Interest in the determinants of benign melanocytic nevi stems from the observation that increased nevus number is associated with an increased risk of melanoma in the general population (Holman and Armstrong, 1984; Swerdlow *et al*, 1986; Bataille *et al*, 1996). We and others have reported twin study data that provide good evidence that nevus number is largely under genetic control, which raises the possibility that nevus genes exist which may also be low penetrance melanoma susceptibility genes (Easton *et al*, 1991; Zhu *et al*, 1999; Wachsmuth *et al*, 2001).

Sunlight exposure is of interest as the major modifiable environmental risk factor for melanoma (Armstrong and Kricker, 1993; Fears *et al*, 2002). There has also been some published evidence for an effect of sun exposure on nevus number, so that nevus number might be useful both as a marker of melanoma risk and of sun exposure within populations. Kelly found that the rate of emergence of nevi increased with proximity to the equator in 1123 Australian schoolchildren aged 6–15 y (Kelly *et al*, 1994). The authors acknowledged that this might represent a cohort effect and there were concerns about low recruitment rates in older age groups. They, however, conclude that there is a relationship between sun exposure and nevi, although genetic effects seem to predominate as the individual variation in nevus density within locations was greater than that between locations. Harrison *et al* (1999) showed higher numbers of nevi in body sites exposed more consistently to the sun in younger Australian children. Similar observations have also been made in European children (Fritschi *et al*, 1994; MacKie *et al*, 1997; Autier *et al*, 1998; Carli *et al*, 1998; Dulon *et al*, 2002).

The relationship between melanoma incidence and sun exposure has meant that sun protection has formed the basis for most primary skin cancer prevention campaigns (Bastuji-Garin and Diepgen, 2002). Recent campaigns emphasize sun avoidance and covering with clothes, rather than an over-reliance on sun protection cream (Vainio *et al*, 2000). This caution has emerged in part because of reported positive correlations between sun protection cream usage and melanoma risk (Garland *et al*, 1992; Donawho and Wolf, 1996; Autier *et al*, 1999, 2000) and in part because sunbathers commonly fail to apply sun protection cream in sufficient quantity (Bech-Thomsen and Wulf, 1992). Furthermore, traditional ultraviolet UVB sun protection creams allow greater time in the sun without burning, thus potentially increasing UVA exposure, which might increase melanoma risk if UVA is also causal for melanoma (Setlow *et al*, 1993; Wang *et al*, 2001).

As nevus number is a significant risk factor for melanoma, the effect of sun protection measures on the nevus phenotype (as a surrogate for melanoma) is of interest. Sun avoidance education and low-cost protective swimwear were shown to reduce exposure, tanning, and nevus counts (by 3%–11%) in a Perth study, although this did not reach statistical significance (Milne *et al*, 2001, 2002). An earlier prospective, randomized trial in Vancouver also showed fewer new nevi developing in children using factor 30 sun protection cream (average number of new nevi over 3

Abbreviations: BSA, body surface area; DZ, dizygous; MZ, monozygous; PCR, polymerase chain reaction; UV, ultraviolet y = 24) compared with controls (average number new nevi = 28, p = 0.048) (Gallagher *et al*, 2000). In contrast, however, Autier *et al* (1998) in a retrospective observational study showed that in European children of similar age, sun protection cream use was associated with the development of increased numbers of nevi. They postulated that this might be because of higher sun exposure, resulting from longer periods of exposure in children consistently wearing high sun protection factor (SPF) sunscreens and therefore not getting burnt. We have addressed this issue in our study and compared the effects of reported wearing of protective clothing.

In summary, benign nevi are of interest from the point of view of a relationship to melanoma and sun exposure, the only modifiable risk factor for melanoma. This study allows us to quantify the role of sun exposure in a group of individuals where the degree of genetic influence on nevus number is already known (Wachsmuth *et al*, 2001).

Results

In general, analyses were performed on nevus densities unless otherwise stated. All analyses were performed initially for data relating to nevi $\geqslant$2 mm size, but were repeated including nevi $<$2 mm to allow comparison with some other studies. Results are not shown for all nevi but in all cases the observations were similar to those for nevi $\geqslant$2 mm.

Age and sex The twins ranged in age from $10\frac{1}{2}$ to 18 y (average 14 y 4 mo). Regression analysis between age and nevus count showed an increase in count with age of 3 nevi per y (p = 0.02) for nevi $\geqslant$2 mm in size adjusted for phenotype and beach exposure. This trend, however, was not significant for nevus densities and therefore the accumulation of nevi with age in this age group appears to be proportional to body growth.

The sex distribution of the twins was as follows: 84 male monozygous (MZ), 72 male dizygous (DZ), 128 female MZ, 48 female DZ, and 110 female/male DZ. Boys had 13.3 more nevi ($\geqslant$2 mm in size) than girls (95% confidence interval (CI) 7.4–19.3, adjusted for phenotype and beach exposure). In considering nevus densities, boys had 9.3 per m^2 more nevi (95% CI 4.8–13.8 again adjusted for phenotype and beach exposure).

Phenotype In general, individuals with blue eyes and fairer skin types had more nevi than those with darker phenotypes. The association with hair color was, however, less clear, with similar counts for all hair colors apart from a small number of individuals with black hair who had fewer nevi: this did not reach statistical significance (Table IV).

Sun exposure Seventy-seven percent of twins had been on holiday to countries hotter than the UK. The average number of hot holidays was 6 (range 1–20) for a mean duration of 2 wk. Five percent of twins had lived in a sunny climate, median duration 117 wk (range 13–208). During hot holidays, 67% spent time on the beach almost every day for an average of 4 h. In terms of active sunbathing 35% sunbathed almost every day for an average of 2 h. For holidays in the UK, 68% visited the beach at most once or twice per holiday, for an average of 2 h and 76% sunbathed only once or twice per holiday for an average of less than 1 h. Therefore a majority of twins had some high intensity sun exposure and there appeared to be a behavioral pattern toward more sun seeking when abroad than when at home.

Females reported sunbathing significantly more often than males, with 49% of females sunbathing every day compared with 20% of males ($\chi^2 = 36.3$ (3 df), $p < 0.0001$). Furthermore, females spent longer sunbathing per day than did males. Of those males who did sunbathe, 65% sunbathed for less than 1 h at a time compared with only 38% of females ($\chi^2 = 29.0$ (3 df), $p < 0.0001$). There were no significant differences in the sun-exposure measures between males and females at the beach, when engaged in water activities, other activities in the sun or during normal weekends.

For hot holidays the times spent in sun-related activities were highly correlated. This was less true of holidays within the UK. When comparing the behavior abroad to that in the UK our data implied that twins who had rarely travelled spent more time on UK beaches, as one might expect (data not shown).

Table I shows that nevus densities increased significantly with increased sun exposure in hot countries. For example, subjects reporting over 10 exposure days on a beach in a hot country had a nevus density 14.8 per m^2 greater than those with no exposure ($p < 0.0001$). Similar results were found for water and other outdoor activities (data not shown). By contrast, a smaller non-significant trend existed for nevus densities and UK sunbathing with no apparent trend for time on UK beaches, other outdoor holiday activities, or normal weekend exposure. These analyses used two separate models, which may be subject to confounding by the more dominant effect of exposure abroad, since those twins spending more time abroad spent less time on holiday in the UK. Reassuringly, similar results were obtained in a single analysis including exposure in the UK and abroad in one model and in a smaller analysis of the 100 individuals who had never been abroad (data not shown).

Table I also shows a positive correlation between nevus density and hot holiday sun exposure for both continuously and intermittently exposed skin. Figure 1 shows graphically that the size of this effect was similar for continuously exposed and intermittently exposed sites, although overall nevus densities were higher in the continuously exposed sites. In a separate analysis of non-exposed sites (i.e. buttocks) a small positive Spearman's correlation was found between days spent on the beach and nevus number (0.14, p = 0.003).

Sun protection: sun protection cream and clothing Sun protection cream use was reported more consistently when the twins were holidaying in hotter countries as fifty-nine percent reported always applying sun protection cream when on beaches abroad compared with 27% on UK beaches ($\chi^2 = 74.1$ (1 df), $p < 0.0001$). Sun protection cream was infrequently applied during summer school-term times (between May and July) with only 4% of twins using sun protection cream always and 52% never applying sun protection cream during the school week. During their time

Table I. Regression analysis of nevus density and sun exposure: (1) holiday exposure abroad, (2) holidays in the UK, and (3) comparing normally continuously exposed *versus* intermittently exposed sites and exposure abroad

Sun exposure category	Number of individuals	Nevus density (per m^2)[a]	Regression coefficient (95% CI)[b]	p-value[c] for trend
Hot beach = 0 d	103[d]	24	Baseline	
Hot beach <3 d	104	32	7.2 (0.2–14.2)	
Hot beach = 3–10 d	112	35	11.1 (3.9–18.3)	
Hot beach >10 d	104	41	14.8 (7.2–22.4)	0.0002
Sunbathing abroad = 0 h	95[d]	23	Baseline	
Sunbathing abroad <6 h	111	32	9.5 (2.5–16.6)	
Sunbathing abroad 6–72 h	105	38	13.5 (6.0–20.9)	
Sunbathing abroad >72 h	109	37	15.3 (7.6–22.9)	0.0003
UK beach = 0 d	109	32	Baseline	
UK beach ⩽5 d	105	30	3.5 (−2.3 to 9.3)	
UK beach >5⩽18 d	121	34	2.1 (−3.4 to 7.5)	
UK beach >18 d	89	33	4.0 (−2.3 to 10.3)	0.30
Sunbathing UK = 0 h	169	31	Baseline	
Sunbathing UK ⩽72 h	116	32	−0.1 (−4.9 to 4.8)	
Sunbathing UK >72 h	139	35	4.8 (−0.7 to 10.3)	0.10
Continuously sun exposed				
Hot beach = 0 d	103	29	Baseline	
Hot beach <3 d	104	39	8.2 (−0.8 to 17.3)	
Hot beach = 3–10 d	112	40	11.8 (2.6–21.1)	
Hot beach >10 d	104	43	13.2 (3.4–23.0)	0.01
Intermittently sun exposed				
Hot beach = 0 d	103	20	Baseline	
Hot beach <3 d	104	27	6.5 (0.2–12.8)	
Hot beach = 3–10 d	112	31	10.3 (3.9–16.7)	
Hot beach >10 d	104	39	16.1 (9.3–22.9)	<0.0001

[a]Arithmetic means of nevus density (nevi ⩾2 mm size) from raw data.
[b]Regression coefficient adjusted for age, sex, skin type, and three categories each of hair and eye color and random effects model.
[c]p-value from a likelihood ratio test for the effect of sun exposure treated as a numerical value corresponding to ordered categories. Separate regression analyses were performed for hot beach and sunbathing exposure.
[d]Includes 95 individuals who had never been abroad in baseline category.
CI, confidence, interval.

abroad a majority of twins, 63%, used sun protection cream with SPF > 15 compared with 47% in the UK.

Table II shows greater nevus densities in individuals reporting always using high (>15) SPF creams than in those reporting occasional use of low (<10) SPF creams. This effect remains after adjustment for age, sex, eye and hair color, reported skin type, and sun exposure (time on hot beaches).

Table III shows no effect of reported shirt wearing during holidays on nevus density of covered skin (i.e. chest, abdomen, and back = 26% of body surface area). Similar lack of protection by shirt wearing was seen for total nevus counts (data not shown). There was no effect of wearing a hat on nevus count on the face (data not shown).

Heritability analysis The main influence on variation of nevus density between individuals is genetic which is estimated to account for 65% (standard error 11%) of the total variance. This was derived from a variance components analysis of log nevus density (including nevi <2 mm in size, for comparability with Wachsmuth *et al*, 2001), including age and sex as covariates. The remaining 35% was attributable to age and sex (3%), environmental factors shared between the twins (26%, SE 11%), and individual-level environmental factors including measurement error (6%).

Of the variance attributable to genetic effects, 7% of the total variance was associated with eye color, 6% with hair color, and 1% with reported skin type when each factor is considered singly. Surprisingly, these factors combined still accounted for 14% of the total variance. We might have expected some overlap when combining these pigmentary and skin-type characteristics as they tend to be correlated within lighter and darker phenotypes. This leaves 51% of

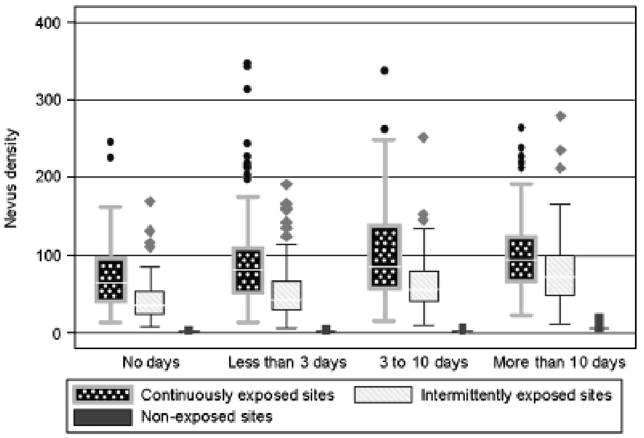

Figure 1
Effect of hot holiday beach exposure on nevus densities (unadjusted) in normally continuously, intermittently, and non-exposed body sites.

the total variance associated with as yet unidentified genetic factors not captured by these phenotypes. Of the 26% of variation attributable to environmental influences, one-third is because of sun exposure on hot holidays.

Discussion

Higher numbers of benign nevi are associated with an increased risk of melanoma. We therefore designed a twin study to enhance our understanding of the genetic and environmental determinants of such nevi.

Previously we have reported the main influence on the development of nevi to be genetic (Wachsmuth *et al*, 2001). In this paper, we have also shown an association between nevus density and reported sun exposure in the same teenage twins. This relationship, however, only reached statistical significance when considering exposure abroad in countries hotter than the UK, which is similar to the observation of Dulon in a study of German schoolchildren, where increased nevus counts were found in children with a history of holidays in hot climates compared with those without (Dulon *et al*, 2002). The implication therefore is that the nevogenic effect of UV is concentration dependent, and more readily demonstrable in areas of higher ambient UV.

Figure 1 shows the relationship between nevus densities in usually continuously, intermittently, and non-exposed body sites and hot holiday beach exposure. Although in general nevus densities were higher in continuously ex-

Table II. Effect of sun protection cream use while on hot holiday on total nevus density

Sun protection cream application	Number of individuals[a]	Mean nevus density[b]	Regression coefficient adjusted for skin type[c] (95% CI)	Regression coefficient adjusted for sun exposure[d] (95% CI)	Regression coefficient adjusting for both skin type and sun exposure[e] (95% CI)
Always use SPF > 15	126	41	Baseline	Baseline	Baseline
Always use SPF 10–15 or most times use SPF > 15	83	32	−4.8 (−10.9 to 1.2)	−6.1 (−12.2 to 0.0)	−5.3 (−11.4 to 0.8)
Sometimes use any SPF or always use SPF < 10	73	29	−8.2 (−15.3 to 1.2)	−10.4 (−17.4 to 3.3)	−8.3 (−15.3 to 1.3)
Never	14	31	−1.9 (−14.0 to 10.1)	−6.6 (−18.8 to 5.6)	−1.8 (−14.1 to 10.5)
p-value			0.07	0.009	0.07

[a]n = 296, i.e. twins who had spent time abroad and reported the SPF of their sun cream.
[b]Arithmetic means of nevus density (nevi ⩾2 mm size) from raw data.
[c]Regression coefficient from random effects model adjusted for age, sex, skin type, and three categories each of hair and eye color.
[d]Regression coefficient from random effects model adjusted for age, sex, three categories each of hair and eye color and sun exposure (time on hot beach).
[e]Regression coefficient from random effects model adjusted for age, sex, skin type, three categories each of hair and eye color and sun exposure (time on hot beach).
p-value from a likelihood ratio test for the effect of sun protection cream treated as a numerical value corresponding to ordered categories.
SPF, sun protection factor; CI, confidence interval.

Table III. Effect of wearing a shirt while on hot holiday on density of nevi of chest, abdomen, and back

Frequency of wearing a shirt on the beach	Number of individuals	Mean total nevus density (⩾2 mm size)[a]	Mean nevus density on trunk (⩾2 mm size)[a]	Regression coefficient[b] (95% CI)
Never	45	35	30	Baseline
Sometimes	179	36	29	−0.9 (−7.5 to 5.6)
Most times	69	36	29	−0.3 (−8.0 to 7.3)
Always	32	33	29	0.9 (−7.8 to 9.6)
			p-value for trend	0.80

n = 325.
[a]Arithmetic means of nevus densities (nevi ⩾2 mm size) from raw data.
[b]Regression coefficient for analysis on trunk nevi adjusted for age, sex, skin type, and three categories each of hair and eye color, sun exposure (time on hot beach) and random effects model.
CI, confidence interval.

posed sites (as one might expect), beach exposure had the effect of increasing densities similarly in both continuously and intermittently exposed sites, the latter presumably becoming more exposed in the holiday beach setting.

That nevus densities are lower on intermittently *versus* continuously exposed body sites suggests that everyday clothing is protective. We were, however, unable to demonstrate a protective effect of shirt wearing on the beach (Table III). It is possible that this simply reflects the unreliable reporting of shirt wearing. Alternatively, shirts worn on holiday may not offer the same degree of UV protection as clothing worn at other times of the year. This view is not supported by Diffey's observation that almost 90% of summer clothing has a UV protection factor (UPF) in excess of 10 (Diffey, 2001). We therefore conclude in our study on nevus development that there is no demonstrable protective effect of shirt wearing while on holiday in hot climates.

With regard to sun protection cream, our results are similar to those of Autier *et al* (1998) who also reported an apparent increase in nevus density with increased application of protection cream. Table II shows this trend to remain after adjustment for skin type and sun exposure. The sun-exposure adjustment was made on the basis of holiday beach exposure in hot countries, which was found to have the strongest nevogenic effect in our study. We assume therefore that if it were possible to perfectly adjust for UV exposure, this trend might disappear and conclude that we have not demonstrated a protective effect of applying sun protection cream.

We have therefore shown that the development of benign nevi is controlled in part by sun exposure above a temperate climate level. Of concern is that we have confirmed the findings of Autier *et al* that adolescents who report the regular use of high SPF sun-protective creams have more nevi than those who do not and that this appears to be independent of skin type. We have therefore provided support for the view that high SPF creams may be nevogenic, putatively by allowing young people to stay in the sun longer. Our aim therefore in skin-care campaigns should remain to emphasize the avoidance of the sun, particularly between the hours of 11 a.m. and 3 p.m. at levels of higher ambient UV, rather than promote the use of high SPF creams as the primary protective measure.

Although reported use of clothing to protect the skin was not associated with a protective effect, no increase in nevus number was seen in users. It seems reasonable therefore to continue recommending the use of clothing in addition to sun avoidance.

From the heritability analysis it is estimated that over half the total variability in nevus density is attributable to genetic factors, other than those associated with eye color, hair color, and reported skin type, indicating the likely existence of genes directly involved in nevus development. These genes may be as yet unidentified nevus genes, however it is also possible that known hair and eye color genes (e.g. MC1R and OCA2) have complex functions and that these and other pigment genes may influence nevus density as suggested by Duffy *et al* (2004). One-quarter of the variability was attributable to environmental factors shared between twins, of which a substantial proportion (one-third) was accounted for by reported sun exposure on hot holidays. A key assumption of a classical twin study is that environmental factors are shared to the same extent between MZ and DZ twin pairs. In our data the correlation in sun exposure between MZ twin pairs was somewhat higher than between same-sex DZ twin pairs. For example, Spearman's correlation coefficients were 0.90 and 0.78, respectively, for time spent on a hot beach (although total length of time spent in a hot country was similarly correlated in MZ and DZ twin pairs 0.99 in MZ and 0.96 in DZ pairs). This may have led to an overestimation of genetic contribution. It is, however, not possible to completely disentangle the effects of genes and environment. In particular, it is likely that there are some genetic influences on sun behavior, possibly related to skin type.

Overall, we have shown nevus number to be modulated by sun exposure but it is not appropriate to use this phenotype as a marker of sun exposure because the major determinants are genetic and largely unknown. It remains to be seen whether genes contributing to the development of benign melanocytic nevi represent low penetrance melanoma genes commonly operating in the general population.

Methods

Interview of the twins One hundred and six MZ twin pairs and 115 DZ twin pairs were included in the study. Detailed methodology has been reported elsewhere (Wachsmuth *et al*, 2001). Ethical committee approval was obtained both from MREC (UK multicenter research ethical committee) and local ethical committees within Yorkshire and Surrey and written informed consent was obtained from all parents. Sun exposure was assessed by means of a self-completed questionnaire. Questions were asked about time spent (either living or on holiday) in hotter countries than the UK; how much time was spent on the beach, sun bathing, pursuing outdoor water, or other outdoor activities; how often sun protection cream was applied; and what SPF was used. Similar questions were also asked for holidays in the UK.

Examination of the twins Benign nevi were counted on 20 body sites, including the iris and conjunctivae, buttocks, and anterior scalp. Nevi were sized into <2, 2 to <5, 5 to <10, and ≥ 10 mm categories using circular templates on transparent acetate. Hair and eye color were assessed and categorized by the observer. Hair color was categorized by use of defined hair swatches. Eye color was defined as blue, brown, or other; categories found to be most reliable after reproducibility testing with a series of high-resolution eye photographs. Skin type was assessed by a Fitzpatrick scoring system, modified by the addition of two intermediate groups to be consistent with earlier studies from this group:

Skin-type scoring system

Skin type	Response of untanned skin to 2 h unprotected sun bathing over several sunny days within the UK
I	always burn never tan
II	burn then tan very slightly
III	burn moderately and tan gradually
IV	burn minimally and tan easily
V	rarely burn and tan deeply
VI	never burn and tan deeply

Zygosity determination Zygosity determination was performed on blood samples of all same-sex twin pairs (166 pairs in total) using seven microsatellite markers: six markers known as the Second Generation Multiplex (Forensic Science Service, Birmingham, UK) and one previously reported to be linked to nevus number (D9S942) (Zhu *et al*, 1999). We have, however, recently reported no evidence of linkage to D9S942 in this twin population (Barrett *et al*, 2003). For each twin pair sharing the same alleles at all loci, the probability of dizygosity was calculated based on observed genotypes and estimated population allele frequencies ($p<0.001$ in over 97% of "MZ" twins).

Statistical analyses Nevus counts (excluding eye nevi) were converted to densities by dividing by surface area (height (cm) $\times$ weight (kg)/3600)$^{0.5}$. Continuously sun-exposed sites were defined as face, neck, lower arms, lower legs, hands, and feet; intermittently sun-exposed sites were defined as upper arms, thighs, chest, abdomen, and back; and non-exposed sites were the buttocks. Nevus densities were then calculated using proportions of body surface area defined by a modification of Wallace's rule of nines applicable to an adolescent (McLatchie, 1990).

In the main, linear regression analysis was used to study the effect of phenotype and exposure on nevus densities and counts. As our sample is of twins, the models included twin pair as a random effect to take account of any similarity within the pairs. This is the simplest form of multilevel model as described by Goldstein (1995). Statistical analysis was carried out using Stata statistical software, Release 8 (Stata-Corporation, 2003).

Initially we looked at the relationship between nevus density and sex, age, skin type, hair, and eye color in univariate random effects models. Subsequent analyses were then adjusted for variation because of these phenotypic factors as presented in Table IV, with the exception that the six categories of hair color were summarized into three (red, blond, and brown/black).

We then analyzed the effect of sun exposure and sun protection on nevus densities for the whole body in addition to continuously, intermittently, and non-exposed areas separately. The hours of sun exposure while sunbathing, on the beach, pursuing other sun-related and water activities for each twin was obtained from the questionnaire. For each activity, dividing the number of hours by 24 derived an "exposure days" variable. These values were then grouped into approximate quartiles (numbers rounded up to whole amounts of time) to produce four categorical variables to describe the amount of sun exposure from each activity. This was carried out both for time spent abroad and time spent in the UK on summer holidays. When considering the effect of exposure during hot holidays the twins who had never been abroad and had only UK beach exposure were included in the "no exposure" category. Spearman's correlations between the different activities were examined in an attempt to decide on appropriate measures of sun exposure (data not shown). Linear regression analyses were performed with all sun-exposure variables.

Sun protection strategies assessed were the use of sun protection creams, shirts, and hats. Sun protection by cream was assigned to four categories by a scoring system allowing for both SPF and frequency of application, as shown below:

1. never apply sun protection cream,
2. sometimes apply any SPF protection cream
   or most times apply SPF <15
   or always apply SPF <10,
3. most times apply SPF >15
   or always apply SPF 10–15,
4. always apply SPF >15.

Correlation between sun exposure and buttock nevi was performed using Spearman's correlations, as the numbers of buttock nevi were small and positively skewed.

A heritability analysis of age- and sex-adjusted nevus density using this twin sample was reported previously (Wachsmuth *et al*, 2001). This analysis was repeated including holiday sun exposure, skin type, eye color, and hair color as additional covariates. This was a variance components analysis using Solar (Almasy and Blangero, 1998). Results from this analysis were used to calculate the remaining proportion of variation because of genetic effects and environmental effects, assuming that skin type, hair, and eye color are predominantly genetic and sun exposure environmental.

Table IV. Mean nevus densities according to skin type, eye, and hair color and the significance of variation assessed by separate regression analyses

Phenotype[a]	Number of individuals (%) from raw data	Mean nevus density (per m^2) $\geqslant$2 mm size nevi[b]	95% CI of mean
Blue eyes	174 (39.5)	39	34–43
Green/gray/ hazel eyes	214 (48.5)	31	28–35
Brown eyes	53 (12.0)	19	15–23
$n=441$[c]		$p=0.002$	
Red hair	68 (15.4)	39	32–45
Blond	32 (7.2)	27	19–36
Pale brown	132 (29.9)	31	26–35
Medium brown	102 (23.1)	35	29–41
Dark brown	103 (23.3)	31	26–36
Black	5 (1.1)	12	0.8–23
$n=442$		$p=0.36$	
Skin type I[d]	46 (10.8)	36	28–45
Skin type II[d]	97 (22.7)	42	35–49
Skin type III[d]	132 (30.9)	34	30–38
Skin type IV[d]	95 (22.3)	27	23–30
Skin type V[d]	41 (9.6)	22	17–27
Skin type VI[d]	16 (3.8)	19	11–27
$n=427$		$p=<0.0001$	

[a]Eye and hair color were considered as separate categorical variables, skin type was considered as a numeric variable (scores I–VI).
[b]Arithmetic means from raw data.
[c]Eye color was not possible to categorise in one twin.
[d]Skin type (data not available for 14 twins).
CI, confidence interval.

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References

Almasy L, Blangero J: Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 62:1198–1211, 1998

Armstrong BK, Kricker A: How much melanoma is caused by sun exposure. Melanoma Res 3:395–401, 1993

Autier P, Dore JF, Cattaruzza MS, *et al*: Sunscreen use, wearing clothes, and number of nevi in 6- to 7-year-old European children. European Organization for Research and Treatment of Cancer Melanoma Cooperative Group. J Natl Cancer Inst 90:1873–1880, 1998

Autier P, Dore JF, Negrier S, *et al*: Sunscreen use and duration of sun exposure: A double-blind, randomized trial. J Natl Cancer Inst 91:1304–1309, 1999

Autier P, Dore JF, Reis AC, *et al*: Sunscreen use and intentional exposure to ultraviolet A and B radiation: A double blind randomized trial using personal dosimeters. Br J Cancer 83:1243–1248, 2000

Barrett JH, Gaut R, Wachsmuth R, Bishop JA, Bishop DT: Linkage and association analysis of nevus density and the region containing the melanoma gene CDKN2A in UK twins. Br J Cancer 88:1920–1924, 2003

Bastuji-Garin S, Diepgen TL: Cutaneous malignant melanoma, sun exposure, and sunscreen use: Epidemiological evidence. Br J Dermatol 146 (Suppl. 61):24–30, 2002

Bataille V, Newton-Bishop J, Sasieni P, Swerdlow A, Pinney E, Griffiths K, Cuzick J: Risk of cutaneous melanoma in relation to the numbers, types and sites of nevi: A case–control study. Br J Cancer 73:1605–1611, 1996

Bech-Thomsen N, Wulf HC: Sunbathers' application of sunscreen is probably inadequate to obtain the sun protection factor assigned to the preparation. Photodermatol Photoimmunol Photomed 9:242–244, 1992

Carli P, Biggeri A, Nardini P, De Giorgi V, Giannotti B: Sun exposure and large numbers of common and atypical melanocytic nevi: An analytical study in a southern European population. Br J Dermatol 138:422–425, 1998

Diffey BL: Sun protection with clothing. Br J Dermatol 144:449–450, 2001

Donawho C, Wolf P: Sunburn, sunscreen and melanoma. Curr Opin Oncol 8: 159–166, 1996

Duffy DL, Box NF, Chen W, *et al*: Interactive effects of MC1R and OCA2 on melanoma risk phenotypes. Hum Mol Genet 13:447–461, 2004

Dulon M, Weichenthal M, Blettner M, *et al*: Sun exposure and number of nevi in 5- to 6-year-old European children. J Clin Epidemiol 55:1075–1081, 2002

Easton DF, Cox GM, MacDonald AM, Ponder BAJ: Genetic susceptibility to nevi-a twin study. Br J Cancer 64:1164–1167, 1991

Fears TR, Bird C, Guerry Dt, *et al*: Average midrange ultraviolet radiation flux and time outdoors predict melanoma risk. Cancer Res 62:3992–3996, 2002

Fritschi L, McHenry P, Green A, Mackie R, Green L, Siskind V: Nevi in schoolchildren in Scotland and Australia. Br J Dermatol 130:599–603, 1994

Gallagher R, Rivers J, Lee T, Bajdik C, McLean D, Coldman A: Broad spectrum sunscreen use and the development of new nevi in white children: A randomised controlled trial. J Am Med Assoc 284:2955–2960, 2000

Garland CF, Garland FC, Gorham ED: Could sunscreens increase melanoma risk? Am J Public Health 82:614–615, 1992

Goldstein H: Multilevel Statistical Models. London: Arnold, 1995; 17ff.

Harrison SL, Buettner PG, MacLennan R: Body-site distribution of melanocytic nevi in young Australian children. Arch Dermatol 135:47–52, 1999

Holman CD, Armstrong BK: Pigmentary traits, ethnic origin, benign nevi, and family history as risk factors for cutaneous malignant melanoma. J Natl Cancer Inst 72:257–266, 1984

Kelly JW, Rivers JK, MacLennan R, Harrison S, Lewis AE, Tate BJ: Sunlight: A major factor associated with the development of melanocytic nevi in Australian schoolchildren. J Am Acad Dermatol 30:40–48, 1994

MacKie R, Turner S, Harrison S, MacLennan R: A comparison of the rate of development of melanocytic nevi in infants in Scotland and Australia. Melanoma Res 7:S4, 1997

McLatchie G: Oxford Handbook of Clinical Surgery. Oxford: Oxford Medical Publications, 1990; p 60–61

Milne E, English DR, Johnston R, Cross D, Borland R, Giles-Corti B, Costa C: Reduced sun exposure and tanning in children after 2 years of a school based intervention (Australia). Cancer Causes Control 12:387–393, 2001

Milne E, Johnston R, Cross D, Giles-Corti B, English DR: Effect of a school based sun protection intervention on the development of melanocytic nevi in children. Am J Epidemiol 155:739–745, 2002

Setlow RB, Grist E, Thompson K, Woodhead AD: Wavelengths effective in induction of malignant melanoma. Proc Natl Acad Sci USA 90:6666–6670, 1993

Stata-Corporation: Stata Statistical Software. College Station, TX: Stata-Corporation, 2003

Swerdlow AJ, English J, MacKie RM, O'Doherty CJ, Hunter JAA, Clark J, Hole DJ: Benign melanocytic nevi as a risk factor for malignant melanoma. Br Med J 292:1555–1560, 1986

Vainio H, Miller AB, Bianchini F: An international evaluation of the cancer-preventive potential of sunscreens. Int J Cancer 88:838–842, 2000

Wachsmuth R, Gaut R, Barrett J, *et al*: Heritability and gene environment interactions for melanocytic nevus density examined in a UK adolescent twin study. J Invest Dermatol 117:348–352, 2001

Wang SQ, Setlow R, Berwick M, Polsky D, Marghoob AA, Kopf AW, Bart RS: Ultraviolet A and melanoma: A review. J Am Acad Dermatol 44:837–846, 2001

Zhu G, Duffy D, Eldridge A, *et al*: A major quantitative-trait locus for mole density is linked to the familial melanoma gene CDKN2A: A maximum-likelihood combined linkage and association analysis in twins and their sibs. Am J Hum Genet 65:483–492, 1999