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Genetic and Environmental Influences on Skin Pattern Deterioration

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Sun exposure has been known to cause histological changes in the dermal layer of the skin. Using deterioration in the fine reticular patterning of the epidermal stratum corneum (skin pattern, as measured on the Beagley–Gibson scale) as a proxy measure of histological changes in the dermal layer, previous studies have typically assumed that degradation of skin pattern is largely caused by sun exposure. A twin study comprising 332 monozygotic twin pairs and 488 dizygotic twin pairs at ages 12, 14, and 16 was used to investigate the etiology of variation in skin pattern, particularly in relation to measured sun exposure and skin color. Our results indicate that although self-reported sun exposure is a significant contributor to variation in skin pattern, its effect is small, explaining only 3.4% of variation in skin pattern at age 14. Additive genetic effects explain 86% of variation in skin pattern at age 12 but these effects reduce with age so that 75% of variation is due to additive genetic effects at age 14 and 72% at age 16. This trend of diminishing genetic influences continues into adulthood, with 62% of variation due to non-additive genetic factors in a smaller adult sample (aged 32–86). Skin color explains 10.4% of variation in skin pattern at age 12, which is due to additive genetic influences common to both. Melanin content appears to provide a protective effect against skin pattern deterioration, perhaps because of the structural differences in melanosomes between different skin types or the free radical scavenging properties of melanin.

Key words: melanin/photoaging/epidermis/sunlight.
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Photoaging, or photodamage, are terms used to describe the changes occurring in the skin that result from cumulative exposure to sunlight. There are six histological signs of photodamaged skin that result in a number of conditions including actinic keratoses, solar elastosis, and non-melanotic skin cancer (Cockerell, 2003; Pinnell, 2003; Lewis *et al*, 2004). A histopathological examination to quantify the level of photodamage and, hence, the susceptibility to non-melanotic skin cancer, is expensive and invasive (Fischer *et al*, 1999). An inexpensive and non-invasive proxy to the level of photodamage has been to measure deterioration to the stratum corneum layer of the epidermis. The underlying assumption inherent in this approach is that sunlight causes deterioration in stratum corneum reticular pattern in the same way as it causes photodamage to the dermis. The primary aim of this investigation was to determine the causes underlying the variation in the deterioration of reticular pattern on the stratum corneum (skin pattern) and to assess whether it is a valid proxy measure of photoaging.

One method of scoring impressions of the stratum corneum is the Beagley–Gibson system, which has been used in two large epidemiological studies to date (Holman *et al*, 1984; Green, 1991). Both studies found associations between higher rates of skin pattern deterioration measured on the Beagley–Gibson scale, and both skin cancer and solar keratoses. These studies concluded that the deterioration of epidermal skin pattern is highly correlated with sun exposure. Recently, Battistutta (1998) suggested that the Beagley–Gibson measure of epidermal skin pattern deterioration explains between 16% and 21% of histologically assessed photoaging in the form of dermal elastosis. In contrast, Fritschi *et al* (1995) and Seddon *et al* (1992) found that the Beagley–Gibson grade explained approximately 4% of variation in dermal elastosis. Seddon *et al* (1992) concluded that variation in stratum corneum patterning was indicative of intrinsic skin aging rather than photoaging.

We aimed to determine whether skin color and sun exposure are the major sources of variation in epidermal skin pattern just as they are the major genetic and environmental influences on changes in the dermal layer. Adolescents were preferred because of their greater level of outdoor activities and sun exposure. As cutaneous changes are cumulative in nature, data from a small sample of adult twins were collected. Here, the twin design was used to decompose variation in skin pattern into genetic and environmental components. When modelling a genetic trait under the classical twin design, we expect that genetically identical twins (monozygotic) will have a greater correlation with their co-twins than non-identical (dizygotic) twins (Plomin, 1986; Neale and Cardon, 1992). Data were collected at ages 12, 14, and 16, allowing for changes to the genetic and environmental contributions to epidermal skin pattern during adolescence to be investigated. Skin color and sun exposure data were collected between the ages of 12 and 14.

Sun exposure is involved in changes to the dermis and epidermis such as the development of melanoma and its precursors, squamous cell carcinomas, actinic keratoses,

multiple sclerosis and, to a lesser extent, basal cell carcinomas (Kennedy *et al*, 2003; Milan *et al*, 2003; van der Mei *et al*, 2003). As melanocytes and keratinocytes are the primary components of the epidermis (Taylor, 2002), histological differences in melanocytes and melanosomes may influence the structure of the epidermis and, hence, the rate of skin pattern deterioration. Melanin pigmentation is known to mitigate the effect of sun exposure on the dermis, providing a hundred-fold photoprotective effect against nonmelanoma skin cancer for people with dark skin (Kollias *et al*, 1991). In addition to determining whether any of the genetic or environmental effects on skin color influence epidermal skin pattern, we investigated whether melanin provides a protective effect against skin pattern deterioration. As a preliminary analysis, we determined the proportion of genetic and environmental contributions to variation in skin color, epidermal skin pattern, and sun exposure.

Results

Reliability To ascertain the reliability of the reports and measures, 33 twin pairs were reassessed using the same protocol 6 wk after the first visit. Test–retest correlations for the sun exposure and skin color variables are shown in Table I and indicate that the measures are reliable. Additionally, 50 skin pattern impressions produced during the age 14 visit were rescored after an interval of 9 mo to obtain an estimate of intra-rater measurement error. The skin pattern measure was found to be reliable, with a polychoric correlation of 0.87 (95% confidence intervals: 0.66, 0.97). The point estimate for measurement error of skin pattern is then 0.13 (i.e., 1–0.87), which is entirely encompassed in the estimate for unique environmental influences.

Factor analysis of the sun exposure and skin color variables The optimal way to determine the underlying latent variable for a trait is to construct a measurement model that estimates loadings from a latent variable to multiple observed measures of a trait. The genetic and environmental components of variance can then be determined for that latent variable (Kendler *et al*, 1987). However, this is tedious, if there are a large number of observed variables, as is the case here for sun exposure and skin color. A quick alternative is to estimate factor scores on the assumption of a common pathway model. Then, genetic analysis can be performed on the resulting latent variables, although details in the causality of covariation between measures may be lost. The primary benefit of the latter approach is a faster computational time as there are fewer parameters being simultaneously estimated. Variation in skin color is not limited to the three categories in the questionnaire (Taylor, 2002), and by performing a factor analysis, the dimensionality of the data is captured. Principal factor analysis with Varimax rotation of the nine variables described above (Table I) was performed in LISREL (release 8.30; Jöreskog and Sörbom, 1995) to obtain the covariate subscales. Applying Cattell's scree test (Cattell, 1966), there were two prominent factors, both with an eigenvalue greater than 1, which may be easily identified as a skin color (eigenvalue = 2.80) and sun exposure (eigenvalue = 1.97) factor, respectively (Table I). As some questions were unanswered, listwise deletion resulted in 661 complete twin pairs (of 696) with data for both skin color and sun exposure at age 12 (Table II).

Threshold parsimony Of the 32 tests conducted to determine threshold parsimony, there were two anomalous results. This is to be expected considering the number of tests conducted and the small sample sizes for some

Table I. Assessments of sun exposure and skin color included in the factor analysis, loadings on the two factors with eigenvalues >1, and test–retest reliability

Variable	Description of report or measure	First common factor (skin color)	Second common factor (sun exposure)	Reliability[a]
SUEXWEEK	Sun exposure experienced by the twin during the week. Mean of the four reports between ages 12 and 14	0.12	**0.38**	—
SUEXWKEND	Sun exposure experienced by the twin during the weekend. Mean of the four reports between ages 12 and 14	0.06	**0.64**	—
HRSCHOOL	Twin self-report of sun exposure during school hours as part of the questionnaire at age 12	0.00	**0.59**	0.85
HRSWKEND	Twin self-report of sun exposure during weekends as part of the questionnaire at age 12	0.04	**0.73**	0.94
OWNSKCOL	Twin self-assessed skin color at age 12	**0.82**	0.06	0.99
COTWSKCOL	Assessment of skin color by co-twin at age 12	**0.77**	0.04	1.00
REFLECTAN	Reflectance measure of the dorsum of the left hand at age 12. The average of three measures recorded	**−0.49**	−0.12	0.81
REFLECNAT	Reflectance measure of the inner upper arm at age 12. The average of three measures recorded	**−0.63**	−0.06	0.73
NURSKCOL	Nurse assessment of skin color of the inner upper arm as part of the age 12 visit	**0.87**	0.06	1.00

Bold if factor loading $> |0.3|$.
[a]Based on the test–retest measures of 33 twin pairs assessed 6 wk apart.

Table II. Number of complete twin pairs with data for skin patterning and the factors relating to skin color and sun exposure

	mzff	*mzmm*	*dzff*	*dzmm*	*dzfm*	*dzmf*	Total
Skin pattern age 12 only	25	31	26	25	13	19	139
Age 14 only	1	3	0	0	3	4	11
Age 16 only	32	29	11	9	10	15	106
Ages 12 and 14	90	86	55	72	55	51	409
Ages 12 and 16	0	0	0	0	3	2	5
Ages 14 and 16	1	0	1	1	2	2	7
All 3 ages	18	16	35	26	21	27	143
Total skin pattern	167	165	128	133	107	120	820
Total age 12 skin pattern	133	133	116	123	92	99	696
Total at age 14	110	105	91	99	81	84	570
Total at age 16	51	45	47	36	36	46	261
Total adult skin pattern	59	30	35	13	12	9	158
Skin color and sun exposure factors	129	127	108	112	94	91	661

mzff, monozygotic females; *mzmm*, monozygotic males; *dzff*, dizygotic same-sex females; *dzmm*, dizygotic same-sex males; *dzfm*, dizygotic opposite sex with females firstborn; *dzmf*, dizygotic opposite sex with males firstborn.

variables. Under the most parsimonious threshold models for skin pattern at all ages, males had a greater level of skin pattern deterioration (H4t). An age regression in the adult sample was significant ($\chi^2_1 = 53$, $p < 0.0001$), confirming that epidermal reticular patterning becomes less well defined with age (Lavker *et al*, 1980; Table III). Males reported a greater level of exposure to sunlight than females under the most parsimonious model for the sun exposure factor score (H4t). For the skin color factor score, there was no difference in the distribution of thresholds between males and females (H5t).

Heterogeneity of twin pair correlations Twin pair polychoric correlations for each zygosity group were estimated using Mx (1.57a) for all variables. Significant twin pair correlations established that skin pattern deterioration as well as the skin color and sun exposure factor scores run in families (Table IV). For skin color and skin pattern at ages 12 and 14, the correlations between monozygotic twin pairs are significantly higher than the correlations between dizygotic twin pairs (H2c), suggesting that genetic factors are one of the sources of familial aggregation. For skin pattern at age 16, the monozygotic and dizygotic twin correlations could be equated, which may be due to the small sample size and resulting lack of power. Because the adult sample is small, only correlations pooled across zygosity are shown in Table IV, although these have been corrected for mean differences between sexes and a regression on age.

Genetic modelling of epidermal reticular patterning The dizygotic twin pair correlation for the skin pattern scores was greater than half the monozygotic twin pair correlation in all three adolescent skin pattern data sets (Table IV), suggesting that common environmental influences (C) explain more of the variance than non-additive genetic effects (D) during adolescence. A model containing additive genetic, common environmental, and unique environmental variance (ACE) was fitted to the data for adolescent skin pattern. At ages 12, 14, and 16, common environmental influences could be removed as a source of variation without a significant drop in fit of the model (Table V). The most parsimonious models (AE) suggest that the source of familial aggregation in skin pattern is entirely due to additive genetic influences, which decline from 86% at age 12 to 75% at age 14 and 72% at age 16. Measurement error contributes to 93% of the estimated unique environmental

Table III. Percentage of individuals for each of the categories in the Beagley–Gibson measure of skin patterning at ages 12, 14, 16, and in an adult sample (age 32–86) before recoding

	Female							Male						
	N	1	2	3	4	5	6	N	1	2	3	4	5	6
Age 12	696	1.7	12.6	54.0	28.6	2.0	0	716	0.4	8.5	48.8	37.9	2.1	0.3
Age 14	573	1.0	8.3	50.5	38.2	1.4	0	581	0	3.6	41.3	47.0	5.9	0.8
Age 16	290	0	1.0	39.2	55.1	2.4	0.3	255	0	0	29.2	55.2	6.9	0.7
Adult	313	0.1	5.1	36.4	48.9	6.7	1.9	194	0	1.5	30.4	55.2	9.3	3.6

Includes unpaired twin singletons.

Table IV. Maximum likelihood estimates of twin pair polychoric correlations and 95% confidence intervals for Beagley–Gibson scales, skin color, and sun exposure factors by zygosity group under the indicated threshold (t) and twin pair correlation (c) models

	H4t								H5t		H4t	
	Skin pattern age 12		Skin pattern age 14		Skin pattern age 16		Skin pattern adult		Skin color factor score		Sun exposure factor score	
	r	95% CI	r	95% CI	r	95% CI	r	95% CI	r	95% CI	r	95% CI
H0c												
mzff	0.87	(0.78, 0.93)	0.64	(0.47, 0.76)	0.68	(0.33, 0.89)	—[a]	—	0.96	(0.93, 0.97)	0.73	(0.63, 0.81)
mzmm	0.84	(0.74, 0.91)	0.83	(0.72, 0.90)	0.67	(0.23, 0.90)	—	—	0.96	(0.94, 0.97)	0.96	(0.93, 0.97)
dzff	0.46	(0.27, 0.62)	0.42	(0.17, 0.61)	0.47	(0.02, 0.79)	—	—	0.40	(0.19, 0.56)	0.78	(0.69, 0.84)
dzmm	0.56	(0.37, 0.70)	0.35	(0.10, 0.54)	0.37	(−0.12, 0.75)	—	—	0.31	(0.10, 0.48)	0.59	(0.44, 0.71)
dzfm	0.45	(0.23, 0.63)	0.33	(0.07, 0.54)	0.36	(−0.16, 0.76)	—	—	0.40	(0.22, 0.54)	0.66	(0.52, 0.76)
dzmf	0.41	(0.21, 0.57)	0.56	(0.34, 0.72)	0.66	(0.22, 0.91)	—	—	0.27	(0.03, 0.47)	0.33	(0.12, 0.51)
H2c												
mz	0.86	(0.79, 0.91)	0.74	(0.65, 0.82)	0.68	(0.42, 0.85)	0.63	(0.40, 0.79)	0.96	(0.94, 0.97)	—[b]	—
dz	0.47	(0.37, 0.56)	0.41	(0.30, 0.52)	0.47	(0.24, 0.67)	0.06	(−0.25, 0.35)	0.35	(0.25, 0.44)	—	—

For N, see Table II.
[a]Pooled monozygotic and dizygotic correlations are provided because of the small sample size.
[b]Pooled correlations are not given because of significant heterogeneity between sexes and zygosity.

variance at age 12, suggesting that the proportion of genetic influences at age 12 may be greater than 86%. It is possible that common environmental effects influence skin pattern but that there is insufficient power to detect the effect. For this reason, confidence intervals for the estimates from a saturated ACE model have been included in Table V; these show that shared environmental influences could account for up to 27% of variance at age 12, 31% at age 14, and 67% at age 16.

For adult skin pattern, the dizygotic twin pair correlation was less than half the monozygotic twin pair correlation, suggesting that an ADE model would best fit the data. With so few pairs, genetic analysis was carried out by combining the monozygotic pairs and combining the dizygotic pairs. Although hypotheses concerning twin pair correlations suggested that there may be some sex limitation in adult skin pattern, there was not enough power to detect its influence (see Neale *et al*, 1994). The proportion of genetic influences in adult skin pattern, 0.62, is significantly lower than that for age 12 skin pattern (Table V). However, unlike the adolescent cohort, the genetic influences are non-additive. A model with non-additive sources of variation without an estimate for additive sources of variation was not tested as it is implausible to have a dominance effect without an additive effect (Posthuma *et al*, 2003).

Genetic modelling of skin color For skin color, the dizygotic twin pair correlation was less than half the monozygotic twin pair correlation (Table IV), so an ADE model was fitted. Both additive and non-additive genetic effects influence skin color, cumulatively explaining 96% of the variation (Table V).

Genetic modelling of sun exposure The monozygotic and dizygotic twin pair correlations for sun exposure suggest that a general ACE sex limitation model is the most suitable saturated model. This model provides for different proportions of A, C, and E between sexes as well as estimating the correlation between additive genetic effects of males and females (r_g). The most parsimonious model to explain the data was a common-effects sex limitation model where influences common to both sexes account for the phenotypic correlation but the magnitude of these effects is different between sexes (Neale and Cardon, 1992). In females, variation was explained by common and unique environmental influences (Table V). In males, 34% of variation was due to additive genetic influences.

Variation in skin pattern explained by skin color and sun exposure As a preliminary step to analysis of more complex multivariate models, the polychoric correlations between skin pattern and the categorized skin color and sun exposure factors were estimated using maximum likelihood estimation in Mx (1.57a). Sun exposure explained a significant proportion of variation in skin pattern at ages 12 and 14; however, the proportions of variation explained, 0.9% and 3.4%, respectively, are small (Table VI). Generally, skin color explained more variation in skin pattern than sun exposure. Skin color explained significantly more variation in skin pattern at age 16 (20.5%) than at age 14 (6.9%) (Table VI). The direction of the correlation shows that melanin provides a protective effect against skin pattern deterioration.

Variation in adult skin pattern explained by occupation and a propensity to tan A person employed in predominantly outdoor work is expected to have a higher propor-

Table V. Maximum likelihood estimation and fit of genetic models for skin patterning and the factor scores relating to skin color and sun exposure along with standardized estimates of additive genetic, common environmental, non-additive genetic, and unique environmental influences and their 95% confidence intervals

	−2log Likelihood	df	*vs* model	$\Delta\chi^2$	Δdf	p	A	C	D	E
Age 12 skin pattern (based on H4t)										
I. ACE model	2667.55	1406					0.78 (0.56, 0.90)	0.08 (0.00, 0.27)		0.14 (0.09, 0.21)
II. AE model	**2668.24**	**1407**	**I**	**0.69**	**1**	**0.405**	**0.86 (0.80, 0.91)**			**0.14 (0.09, 0.20)**
III. CE model	2714.08	1407	I	46.53	1	0.000				
Age 14 skin pattern (based on H4t)										
I. ACE model	2190.06	1149					0.65 (0.38, 0.81)	0.09 (0.00, 0.31)		0.26 (0.19, 0.35)
II. AE model	**2190.60**	**1150**	**I**	**0.54**	**1**	**0.464**	**0.75 (0.67, 0.82)**			**0.25 (0.18, 0.33)**
III. CE model	2210.75	1150	I	20.69	1	0.000				
Age 16 skin pattern (based on H4t)										
I. ACE model	676.05	541					0.41 (0.00, 0.84)	0.27 (0.00, 0.67)		0.32 (0.15, 0.58)
II. AE model	**677.19**	**542**	**I**	**1.14**	**1**	**0.284**	**0.72 (0.51, 0.86)**			**0.28 (0.14, 0.49)**
III. CE model	677.62	542	I	1.57	1	0.210				
Adult skin pattern (based on H4t with age displ.)										
I. ADE model	**879.45**	**507**					**0.00 (0.00, 0.08)**		**0.62 (0.24, 0.78)**	**0.38 (0.07, 0.61)**
II. AE model	888.23	508	I	8.78	1	0.003				
III. E model	900.85	509	II	12.62	1	0.000				
Skin color factor score (based on H5t)										
I. ADE model	**4355.70**	**1354**					**0.44(0.05, 0.80)**		**0.52 (0.16, 0.91)**	**0.04 (0.03, 0.06)**
II AE model	4363.97	1355	I	8.27	1	0.004				
Sun exposure factor score (based on H4t)										
							A_{female} A_{male}	C_{female} C_{male}	E_{female} E_{male}	r_g^a
I. General sex limitation model	4454.40	1350					0.00 0.34	0.75 0.36	0.25 0.30	0.35
II. Common effects sex limitation model	4454.40	1351	I	0	1	1	0.00 0.34	0.75 0.36	0.25 0.30	0.50
III. Drop A_{female}	**4454.40**	**1352**	**II**	**0**	**1**	**1**	**0.34 (0.16, 0.50)**	**0.75 (0.68, 0.80)** **0.36 (0.22, 0.51)**	**0.25 (0.20, 0.32)** **0.30 (0.22, 0.39)**	**0.50**
IV. ACE model	4468.23	1354	II	13.83	3	0.003	0.22	0.50	0.29	0.50

Bold indicates the most parsimonious model to explain the data.
[a] r_g is the estimated correlation between additive genetic effects of males and females.

tion of cumulative exposure to sunlight over their lifetime. Similarly, a propensity to tan is perhaps indicative of an individual's skin color as people with darker skin color tend to tan rather than burn. A maximum likelihood estimate of the proportion of variation in adult skin pattern explained by the self-report of a propensity to burn and occupation type is shown in Table VI. Both correlations were negative, indicating that an inability to tan and greater time working outdoors were related to greater skin pattern deterioration. But very little variation in adult skin pattern, less than 2%, was explained by either of these reports.

Bivariate analysis of skin pattern and skin color As a relatively large proportion of variation in skin pattern is explained by skin color during adolescence, a bivariate analysis was performed to determine the sources of variation

Table VI. Maximum likelihood estimation of the percentage of variation in skin pattern explained by the factor scores related to sun exposure and skin color in adolescents and by lifetime occupation and a propensity to tan in adults

	Sun exposure	Skin color
Age 12 skin pattern (%)	0.9 (0.1, 2.3)	10.4 (7.1, 14.2)
Age 14 skin pattern (%)	3.4 (1.4, 6.2)	6.9 (4.0, 10.5)
Age 16 skin pattern (%)	2.1 (0.0, 3.2)	20.5 (11.4, 30.6)
	Lifetime occupation	**Propensity to tan**
Adult skin pattern (%)	1.4 (0.0, 6.8)	1.1 (0.0, 5.9)

that mediate the covariation between skin color and skin pattern. A Cholesky decomposition of the sources of variation on skin pattern at age 12 showed that the genes causing the additive genetic effects in skin color also influence skin pattern at age 12 (Fig 1).

Bivariate analysis of skin pattern and sun exposure Although the correlation between sun exposure and skin pattern at age 14 is small (Table VI), the relationship between them may foreshadow the cumulative influence of sun exposure as it increases with age. Modelling of the relationship as a bivariate Cholesky decomposition indicated that estimates of the sources of covariation were different between sexes. In females, there was no significant correlation between sun exposure and skin pattern at age 14 (Fig 2). In males, the correlation was explained by common environmental influences causing variation in both sun exposure and skin pattern at age 14.

Discussion

This was a study aimed at decomposing variation in epidermal reticular patterning (skin pattern) into genetic and environmental influences and estimate the concomitant effect of sun exposure and skin color. The heritability estimate for skin pattern was 86% at age 12 but declined with age to 75% at age 14, 72% at age 16, and 62% in our small sample of adults with a mean age of 47. After taking measurement error into account, less than 2% of variation in skin pattern at age 12 is due to individual environmental influences but this increases to 17.6% by age 16. This may well be the effect of cumulative sun exposure as it increases with age. Little variation in adolescent skin pattern is because of environmental influences and in fact, common environmental influences could be removed as a source of variation. This may be because of a lack of power to detect these influences. For an in-depth discussion of the causes of failure to detect common environmental effects in many studies, see Martin *et al* (1978) and Hopper (2000). In the adult sample, non-additive genetic influences explained all the familial aggregations of skin pattern. This may be because of accentuated gene action or new genes being switched on subsequent to adolescence. When taking into account the results of Lavker *et al* (1980), who found a differential rate of deterioration in skin pattern between exposed and unexposed areas of the skin, our results suggest that genetic influences modify the effect of sun exposure on skin pattern.

Sun exposure, although largely due to common and unique environmental influences, showed a significant proportion of genetic effects in males (but not females), perhaps because of genes that either predispose or cause an aversion to outdoor activity. All the familial variation in females, and 51% in males, was due to common environmental influences. As exposure to sunlight is mediated by common parental and pedagogical influences during adolescence, and these environmental influences are shared between twins, it is surprising that common environmental influences could be removed as a source of variation in skin pattern. Here, measured sun exposure explains between 0.9% (age 12) and 3.4% (age 14) of variation in skin pattern during adolescence. This suggests either that skin pattern is not a suitable proxy measure for photoaging or that our attempts to measure lifetime and current sun exposure have been largely unsuccessful. A bivariate Cholesky analysis between sun exposure and skin pattern at age 14 showed that environmental influences shared between male twin pairs influence both reported sun exposure and skin pattern. This may be because of the social and sporting activities shared between adolescent male twins. Although genetic influences account for the balance of variation in skin pattern of both adolescents and adults, cutaneous changes are progressive, suggesting that a study of twins in their seventh to ninth decades would provide a more accurate estimate of the proportion of lifetime genetic and environmental influences. Leung and Harvey (2002) found that cumulative sun exposure did not influence skin patterning once age was included as a covariate for a sample with a mean age of 71.

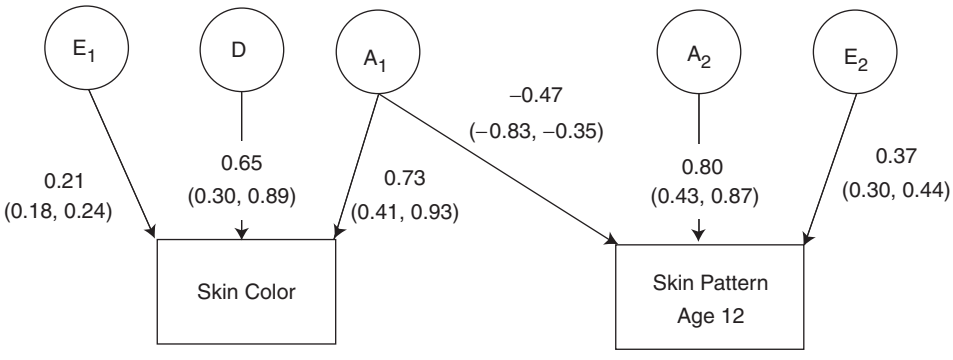

Figure 1
Bivariate Cholesky decomposition of variation in age 12 skin pattern into that influencing skin color and that which is unique.

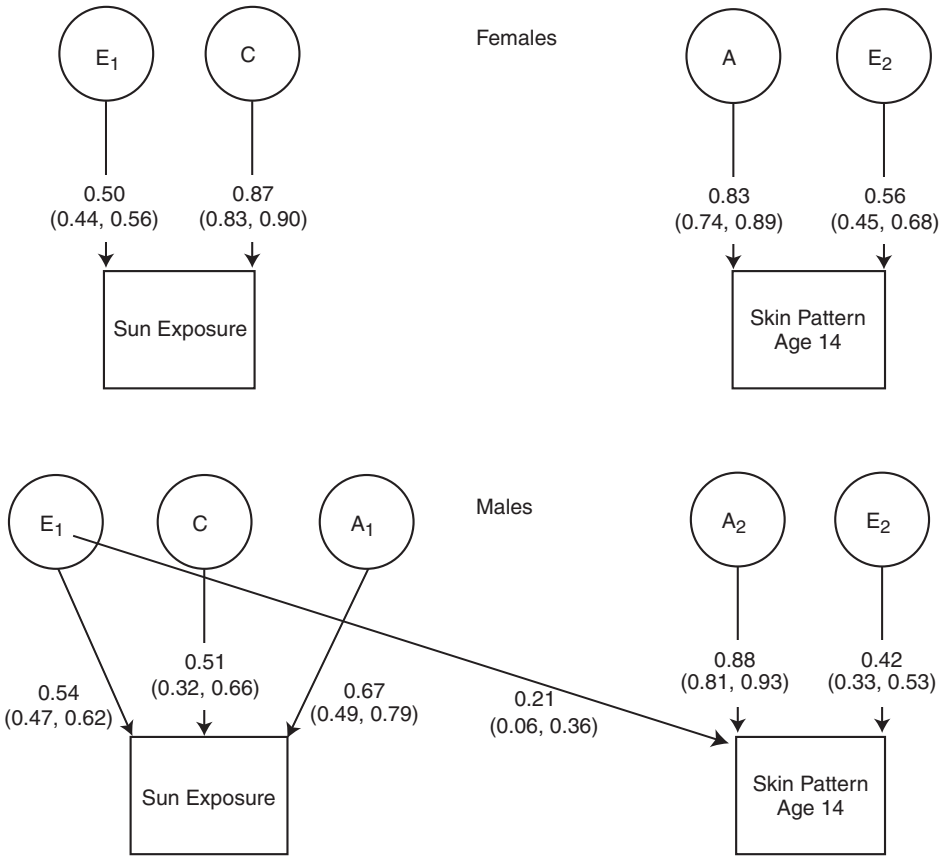

Figure 2
Sex-limited bivariate Cholesky decomposition of variation in skin pattern at age 14 into that influencing skin color and that which is unique. For females, there is no correlation between sun exposure and skin pattern.

The composite skin color variable used in this analysis included reports and measures of both exposed and unexposed areas of the skin. The composite variable showed a heritability of 96% (see Harrison and Owen, 1964; Clark *et al*, 1981; Williams-Blangero and Blangero, 1992 on skin reflectance heritability). These genetic effects were due to additive and non-additive (dominance effects; epistatic interactions) influences. Pigmentation traits such as red hair, fair skin, and a lack of tanning ability are associated with recessive alleles of the MC1R gene (Sturm *et al*, 2003), suggesting that it is a good candidate for the non-additive genetic effects seen in skin color.

Skin color explains 10.4% of the variation in skin pattern at age 12. The additive genetic effects on skin color that also influence skin pattern may be genes for melanocyte formation and aggregation or melanin type and cohesion (Taylor, 2002). Melanin content in the skin (tanned and untanned) is inversely proportional to deterioration in epidermal skin pattern, showing a protective effect of melanin against skin pattern deterioration. The cohesive and free radical scavenging properties of pheomelanin and eumelanin may be the source of this protective effect (see Toda *et al*, 1972; Olson *et al*, 1973; Weigand *et al*, 1974; Scalia *et al*, 1990; Bustamante *et al*, 1993). It is not clear whether dermal elastosis is independent of melanin content (Nurnberger *et al*, 1978; Montagna and Carlisle, 1991). A relationship between dermal elastosis and melanin content could explain the correlation between skin color and skin pattern observed in this study. From our analysis, skin color explains 26% of the genetic influences on skin pattern at age 12, and measured sun exposure explains 2.2% of the familial variation in age 12 skin pattern. Dermal elastosis explains between 4% and 21% of variation in skin pattern (Seddon *et al*, 1992; Fritschi *et al*, 1995; Battistutta, 1998). So much of the variation in skin pattern is still unaccounted for.

Future studies would benefit from recent advances. Dwyer *et al* (2002) have developed a measure of cutaneous melanin density using spectrophotometry, which may be an objective measure suitable for future studies. Increased hemoglobin levels are correlated with clinically perceived skin tanning, requiring it to be taken into account in further analyses using skin reflectance (Stamatas and Kollias, 2004). More objective methods of measuring sun exposure may be suitable for future studies, from a mutation in mtDNA (Krishnan *et al*, 2004) or the use of a wristwatch that measures sun exposure over time (Thieden *et al*, 2004). The MC1R genotypes, which have been associated with skin cancer and skin color (Sturm, 2002), will also be investigated in relation to sun exposure and skin pattern. The large additive genetic variance in adolescent skin pattern suggests that it would be worthwhile performing a linkage analysis to find quantitative trait loci influencing this trait.

Materials and Methods

Population sample The data for the adolescent cohort used in this study were collected as part of a longitudinal study investigating the development of melanocytic nevi (moles). Details of the clinical protocol are described in Zhu *et al* (1999) and McGregor *et al* (1999). Twins were enlisted by contacting principals of primary schools in the greater Brisbane area through word of mouth and a

range of media. The twins who registered their interest were contacted and participation was conditional upon the informed consent of the twins and their parents. Approval to undertake this study was granted by the Human Research Ethics Committee of the Queensland Institute of Medical Research. The study was conducted according to the Declaration of Helsinki Principles. The analysis of skin pattern was based on 820 complete twin pairs collected between May 1992 and December 2003 (see Table II). The protocol included the collection of skin pattern impressions, reports of sun exposure, and measures of skin color.

Skin pattern impressions were obtained from an adult sample in the context of a much larger effort to obtain blood samples from twins in an alcohol study (Heath *et al*, 1997; Whitfield *et al*, 2000). Individuals were also asked about their propensity to tan and whether their occupation involved mainly working indoors or outdoors. The adult data, consisting of 158 complete twin pairs, were collected between September 1993 and February 1995 (Table II). The age ranged between 32 and 86 y with an average of 47.5 ($\pm$ 11) y.

Zygosity testing Zygosity of the adolescent twins was tested by typing the ABI Profiler Plus marker set consisting of nine highly polymorphic DNA microsatellite markers and the amelogenin sex marker at the Queensland Institute of Medical Research (QIMR), Brisbane. Zygosity was assigned with a probability of error less than 10^{-4}. In 33 pairs of twins where DNA was not available, zygosity was based on the similarity of appearance judged by the parents. The zygosity of 55 twin pairs in the adult sample was typed either using the ABI Profiler Plus marker set or through genotyping the individuals. The zygosity of the remaining 82 same sex adult twin pairs was self-reported.

Measures

Measuring deterioration in epidermal reticular pattern A measure of stratum corneum deterioration was produced by using an Affinis light body silicone elastomer (manufactured by Coltène AG, Altstätten, Switzerland) to take an impression of the dorsum of the left hand, which was held in a relaxed position by loosely gripping a cardboard cylinder (for more details, see Sarkany, 1962; Sarkany and Caron, 1965; Barnes, 1973; Battistutta, 1998). The silicone impressions of the skin were scored according to the Beagley–Gibson rating using a low-power dissecting microscope. The Beagley–Gibson rating classifies patterns into six categories depending on the evenness, clarity, and depth of primary and secondary lines on the skin (see Holman *et al*, 1984, for additional details and figures). A higher score indicates greater epidermal skin pattern deterioration. In the adolescent cohort, the twins were tested as close to their 12th, 14th, and 16th birthdays as possible. All silicone impressions were scored by the same individual who was blind to zygosity.

Measures of sun exposure and skin color Measures of sun exposure and skin color were collected for the adolescent twins. A questionnaire was presented to the twins during the session in which skin pattern impressions were taken at age 12 (Zhu *et al*, 1999). This questionnaire contained items regarding skin color and exposure to sunlight (see Table I). Twins were asked *to count how many hours they would spend out in the sun during a normal school week (in summertime)* (HRSCHOOL); *during a normal weekend (in summertime)* (HRSWKEND); *what type of skin color do you think you have?* (OWNSKCOL); and *what type of skin color do you think your twin has?* (COTWSKCOL). The nurse who produced the silicone mold impression also rated skin color on the inner upper arm (NURSKCOL). The possible responses for the skin color questions were: *fair-pale*; *medium*; or *dark/olive*. The possible responses for the sun exposure questions were *less than 2 hours*; *more than 2 hours but less than 5 hours*; *more than 5 hours but less than 10 hours;* and *more than 10 hours*.

In addition to these, continuous reports of sun exposure were collected by mail or telephone interview four times at each intervening April and September between the age 12 visit and the age 14 visit. The question asked: *approximately how much time have you been spending in the sun between the hours of 10 am and 2 pm over the last few months*? A response was requested, in hours and minutes, for each day of a typical school week. A report of sun exposure during the week (SUEXWEEK) and during the weekend (SUEXWKEND) was calculated by averaging the four respective biannual reports.

Skin reflectance Skin reflectance is a measure of the amount of light reflected by the surface of the skin. An *in vitro* study has shown that the melanin content of the skin is linearly proportional to the inverse of the reflectance value (Harrison and Owen, 1956). Skin reflectance has been used in previous epidemiological studies (Clark *et al*, 1981; Green and Martin, 1990; Williams-Blangero and Blangero, 1992). The greatest resolving power to differentiate between different skin colors was at 650 nm, which is also the wavelength for which melanin has the highest absorbance (Harrison and Owen, 1964). An EEL DS29 Unigalvo reflectance spectrophotometer (manufactured by Diffusion Systems Ltd, Hanwell, London, UK) was used to take two measures. Before measurements were taken for each individual, the instrument was calibrated against a white tile (Green and Martin, 1990). A measure of skin reflectance was taken of the back of the left hand (REFLECTAN) as a measure of tanned skin color. Reflectance was also measured from the inner upper arm (REFLECNAT) as an indication of natural, or untanned, skin color. For each site, the average of three measurements was recorded.

Measures in the adult sample At the same time that silicone molds were made, a questionnaire was administered asking if they *were exposed to strong sun for the first time in summer for an hour with no protection, would you: always burn, never tan?; burn, then tan?;* or *only tan?* In addition, twins were asked: *overall, have your occupations been: mainly outdoors?; both indoors and outdoors?;* or *mainly indoors?*

Data analysis

The threshold model The skin pattern data, being ordinal, were analyzed using the multifactorial threshold model (MFT), which assumes that there is a latent variable, or liability, underlying the trait and that ordered categories reflect thresholds imposed on an underlying normal distribution of liability. Correlations under the threshold model take this into consideration such that joint distribution of the underlying scale of liability for skin pattern with liability scales underlying other ordinal variables, and with continuous variables, is bivariate normal (Reich *et al*, 1979; Martin *et al*, 1988).

Data preparation To avoid computational problems in the program Mx (version 1.57a; Neale *et al*, 2002), data were recoded so that there were no categories of skin pattern without individuals for each zygosity. This required the skin pattern data to be recoded from six to four categories at ages 12 and 14 (the first and last two categories were collapsed). At age 16, where there were appreciably fewer data points, the number of categories was reduced to three (first three categories collapsed, last two categories collapsed). The adult sample was reduced to three categories (first two categories collapsed, last three categories collapsed).

To perform a bivariate analysis in Mx between skin pattern and either the skin color or sun exposure common factors (factor analysis described below), the skin color and sun exposure factor scores were polychotomized into six categories. To optimize power, each of these categories had approximately equal numbers (Neale *et al*, 1994). Empty cells in the calculation of the polychoric correlation may bias the goodness-of-fit test. When genetic analyses were run with fewer categories, the parameters estimated were similar to those from analyses with all categories, suggesting that the maximum likelihood estimation of polychoric correlations is not highly sensitive to small cell counts in this instance.

Table VII. Hypotheses for testing threshold and twin pair correlation homogeneity

	Testing of thresholds												Testing of twin pair correlations			
	H0t		H1t		H2t		H3t		H4t		H5t		Tested on the most parsimonious threshold model			
	Twin 1[a]	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	Twin 1	Twin 2	H0c	H1c	H2c	H3c
mzff	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
mzmm	3	4	2	2	2	2	2	2	1+Δ	1+Δ	1	1	2	1	1	1
dzff	5	6	3	3	1	1	1	1	1	1	1	1	3	2	2	1
dzmm	7	8	4	4	2	2	2	2	1+Δ	1+Δ	1	1	4	2	2	1
dzfm	9	10	5	6	3	4	1	2	1	1+Δ	1	1	5	3	2	1
dzmf	11	12	7	8	5	6	2	1	1+Δ	1	1	1	6	4	2	1

[a]Twin 1 indicates first born and twin 2 indicates second born.

Within a model, each number represents a separate free parameter and the equality of numbers indicates that these parameters are constrained equal. The null model, H0t, is a saturated model because every statistic has its own parameter, including separate twin pair correlations for each zygosity group. To test whether there is an effect of birth order, H1t hypothesizes equal thresholds for first born (twin 1) and second born (twin 2) twins within same-sex zygosity groups. H2t tests equality of thresholds within like-sex zygosity groups. H3t tests whether being in the presence of the opposite sex during gestation causes a threshold difference (see Loehlin and Martin, 1998; Loehlin and Martin, 2000). H4t tests whether each male threshold can be explained by a constant displacement (Δ) from its female counterpart. H5t tests whether thresholds of males and females can be equated.

Testing of threshold homogeneity Maximum likelihood analysis was used to test hypotheses relating to the thresholds defining the distribution of skin pattern scores, the skin color factor, and the sun exposure factor (Lange *et al*, 1976; Neale *et al*, 2002). These tests are important to determine the randomness of sampling and to identify possible non-uniform influences on the data. A saturated model was initially fitted to the data, which allowed the parameters estimating threshold values to vary without restriction. The parameters were then equated in a stepwise sequence, with each model nested within the preceding model (Table VII). Twice the difference in log likelihoods between the full and submodels is distributed as χ^2 with the degrees of freedom equal to the difference in degrees of freedom between the two models (likelihood ratio test, Neale and Cardon, 1992).

Hypotheses regarding thresholds that were tested on skin pattern, skin color, and sun exposure are outlined in Table VII (see also McGregor *et al*, 1999; Gillespie *et al*, 2000). For variables with greater than two categories, H4t is an important test of whether there is an underlying normal distribution of liability. If the frequency distribution of responses in one sex can be predicted from normal distribution theory and a simple displacement Δ from thresholds of the other sex, this is strong evidence for the MFT, especially if the number of categories is large (Neale and Cardon, 1992).

Testing the homogeneity of twin pair correlations Twin pair correlations are important in twin studies because they are used to decompose interindividual variation into genetic and environmental components. If the correlation between monozygotic twin pairs is greater than that of dizygotic twin pairs, we assume that the familial aggregation is driven by genetic influences, as monozygotic twin pairs share all their genes, whereas dizygotic twin pairs share roughly half their genes. To determine whether variance within a trait is influenced by genetic factors, the change in fit of H3c from H2c was tested. In non-scalar sex limitation, which can be tested in ordinal data, different genes are influencing the trait for each sex, resulting in a lower correlation between brother and sister (opposite sex) twin pairs than would be predicted from the brother–brother and sister–sister (same sex) twin correlations (Eaves, 1977). Non-scalar sex limitation is tested by the change in fit of H2c from H1c. These tests were carried out on the background of the most parsimonious threshold model for each respective variable.

Genetic modelling Using the known basis for similarity of monozygotic and dizygotic twins, twin pair correlations can be used to decompose the variance of a trait into genetic and environmental influences. Genetic variation can be subdivided into additive (A) and non-additive (D) influences. Additive genetic influences are those where the effect on the trait is the sum of the effects of the alleles influencing the trait. Monozygotic twin pairs are perfectly correlated for additive effects, whereas dizygotic twin pairs, who share roughly half their genes, are expected to correlate about 0.5. Non-additive influences can be allelic interactions (dominance) or non-allelic interactions (epistasis). There is no possibility of distinguishing between dominance and epistasis in a classical twin design so the non-additive term is usually called "dominance," with dizygotic twins expected to correlate 0.25. However, a significant estimate of D may suggest that either dominance, epistasis, or both, are significant contributors to the variation. Variation due to the environment can be modelled as either that influencing both twins (C) or that influencing each twin disproportionately (E). If familial aggregation for a trait is influenced purely by a common environment (C), we expect the twin pair correlation for monozygotic twins to be the same as that for dizygotic twins. By definition, twins are not correlated for unique environmental effects (see Fisher, 1918; Jinks and Fulker, 1970; Eaves, 1977; Mather and Jinks, 1982; Neale and Cardon, 1992; Posthuma *et al*, 2003, for further details on genetic modelling).

Estimates of genetic and environmental influence are made under the underlying assumption that the trait-relevant environmental

influences on monozygotic twins are no more correlated than for dizygotic twins. Where more similar environments are experienced by monozygotic twins, these have been shown to be elicited by their more similar genotypes rather than through imposition of external views of how they should be treated (Loehlin and Nichols, 1976; Plomin *et al*, 1977; Scarr and McCartney, 1983; Martin *et al*, 1986; Kendler *et al*, 1993).The estimates of common environmental influences (C) and non-additive genetic influences (D) are both derived from the relationship between monozygotic and dizygotic twin pair correlations, and are negatively confounded. To be able to estimate A, C, and D in the same model, further relationships are required (e.g., twins reared apart). If the dizygotic twin pair correlation is more than half the monozygotic twin pair correlation, then common environmental (C) influences are a greater source of variation than non-additive genetic (D) effects. When the dizygotic twin pair correlation is less than half the monozygotic twin pair correlation, non-additive genetic effects have more influence.

Based on inspection of the monozygotic and dizygotic correlations, either an ACE or an ADE model was fitted to each variable using Mx (1.57a). Nested models were fitted by dropping A, C, and D in appropriate combinations. The unique environmental parameter, E, includes measurement error so it cannot be dropped from the model. The likelihood ratio test was used to assess the fit of submodels (Neale and Cardon, 1992).

Genetic modelling of the relationship between skin color, sun exposure, and skin pattern A correlation between skin pattern and either skin color or sun exposure can be because of environmental or genetic influences common between the respective traits. A bivariate Cholesky model (Neale and Cardon, 1992) was fitted to decompose variation in skin pattern at age 12 into that because of skin color and that unique to itself. A separate bivariate Cholesky model was used to decompose variation in skin patterning at age 14 into that because of the sun exposure common factor and that unique to itself. Age 14 was used for the latter as the variables that contribute to the sun exposure factor score included reports collected between the ages 12 and 14 visit. The likelihood ratio test was used to determine model parsimony (Neale and Cardon, 1992).

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References

Barnes IE: Techniques for the replication of skin surfaces. A new method. Br J Dermatol 89:277–283, 1973

Battistutta D: Skin Photoageing: Epidemiological Measurement and Determinants. Brisbane: University of Queensland, 1998

Bustamante J, Bredeston L, Malanga G, *et al*: Role of melanin as a scavenger of active oxygen species. Pigment Cell Res 6:348–353, 1993

Cattell RB: The scree test for the number of factors. Multivariate Behav Res 1:245–276, 1966

Clark P, Stark AE, Walsh RJ, *et al*: A twin study of skin reflectance. Ann Hum Biol 8:529–541, 1981

Cockerell CJ: Pathology and pathobiology of the actinic (solar) keratosis. Br J Dermatol 149 (Suppl. 66):34–36, 2003

Dwyer T, Blizzard L, Ashbolt R, *et al*: Cutaneous melanin density of Caucasians measured by spectrophotometry and risk of malignant melanoma, basal cell carcinoma, and squamous cell carcinoma of the skin. Am J Epidemiol 155:614–621, 2002

Eaves L: Inferring the causes of human variation. J R Stat Soc 140:324–355, 1977

Fisher RA: The correlation between relatives on the supposition of Mendelian inheritance. Trans R SOC Edinb 52:399–433, 1918

Fischer TW, Wigger-Alberti W, Elsner P: Direct and non-direct measurement techniques for analysis of skin surface topography. Skin Pharmacol Appl Skin Physiol 12:1–11, 1999

Fritschi L, Battistutta D, Strutton GM, *et al*: A non-invasive measure of photoageing. Int J Epidemiol 24:150–154, 1995

Gillespie NA, Zhu G, Heath AC, *et al*: The genetic aetiology of somatic distress. Psychol Med 30:1051–1061, 2000

Green A: Premature ageing of the skin in a Queensland population. Med J Aust 155:473–474, 1991; 477–478

Green A, Martin NG: Measurement and perception of skin colour in a skin cancer survey. Br J Dermatol 123:77–84, 1990

Harrison GA, Owen JJ: The application of spectrophotometry to the study of skin colour inheritance. Acta Genet Stat Med 6:481–484, 1956; discussion 484–485

Harrison GA, Owen JJ: Studies on the inheritance of human skin colour. Ann Hum Genet 28:27–37, 1964

Heath AC, Bucholz KK, Madden PA, *et al*: Genetic and environmental contributions to alcohol dependence risk in a national twin sample: Consistency of findings in women and men. Psychol Med 27:1381–1396, 1997

Holman CD, Armstrong BK, Evans PR, *et al*: Relationship of solar keratosis and history of skin cancer to objective measures of actinic skin damage. Br J Dermatol 110:129–138, 1984

Hopper JL: Why common environmental effects are so uncommon. In: Spector TD, Sneider H, MacGregor AJ (editor, translator, and editor) Advances in Twin and Sib-pair Analysis. London: Greenwich Medical Media, 2000; p 151–165

Jinks JL, Fulker DW: Comparison of the biometrical genetical, MAVA, and classical approaches to the analysis of human behavior. Psychol Bull 73: 311–349, 1970

Jöreskog KG, Sörbom D: Prelis 2 User's Reference Guide. In: 2.30 end. Series Prelis 2 User's Reference Guide. Chicago: Scientific Software International Inc., 1995

Kendler KS, Heath AC, Martin NG, *et al*: Symptoms of anxiety and symptoms of depression. Same genes, different environments? Arch Gen Psychiatry 44:451–457, 1987

Kendler KS, Neale MC, Kessler RC, *et al*: A test of the equal-environment assumption in twin studies of psychiatric illness. Behav Genet 23:21–27, 1993

Kennedy C, Bajdik CD, Willemze R, *et al*: The influence of painful sunburns and lifetime sun exposure on the risk of actinic keratoses, seborrheic warts, melanocytic nevi, atypical nevi, and skin cancer. J Invest Dermatol 120:1087–1093, 2003

Kollias N, Sayre RM, Zeise L, *et al*: Photoprotection by melanin. J Photochem Photobiol B 9:135–160, 1991

Krishnan KJ, Harbottle A, Birch-Machin MA: The use of a 3895 bp mitochondrial DNA deletion as a marker for sunlight exposure in human skin. J Invest Dermatol 123:1020–1024, 2004

Lange K, Westlake J, Spence MA: Extensions to pedigree analysis. III. Variance components by the scoring method. Ann Hum Genet 39:485–491, 1976

Lavker RM, Kwong F, Kligman AM: Changes in skin surface patterns with age. J Gerontol 35:348–354, 1980

Leung WC, Harvey I: Is skin ageing in the elderly caused by sun exposure or smoking? Br J Dermatol 147:1187–1191, 2002

Lewis KG, Bercovitch L, Dill SW, *et al*: Acquired disorders of elastic tissue: Part I. Increased elastic tissue and solar elastotic syndromes. J Am Acad Dermatol 51:1–21, 2004; quiz 22–24

Loehlin JC, Martin NG: A comparison of adult female twins from opposite-sex and same-sex pairs on variables related to reproduction. Behav Genet 28:21–27, 1998

Loehlin JC, Martin NG: Dimensions of psychological masculinity-femininity in adult twins from opposite-sex and same-sex pairs. Behav Genet 30: 19–28, 2000

Loehlin JC, Nichols RC: Heredity, Environment and Personality: A Study of 850 Sets of Twin Pairs. Austin, TX: University of Texas Press, 1976

Martin NG, Eaves LJ, Heath AC, *et al*: Transmission of social attitudes. Proc Natl Acad Sci USA 83:4364–4368, 1986

Martin NG, Eaves LJ, Kearsey MJ, *et al*: The power of the classical twin study. Heredity 40:97–116, 1978

Martin NG, Jardine R, Andrews G, *et al*: Anxiety disorders and neuroticis: Are there genetic factors specific to panic? Acta Psychiatr Scand 77:698–706, 1988

Mather K, Jinks JL: Biometrical Genetics, 3rd edn. London: Chapman & Hall, 1982

McGregor B, Pfitzner J, Zhu G, *et al*: Genetic and environmental contributions to size, color, shape, and other characteristics of melanocytic naevi in a sample of adolescent twins. Genet Epidemiol 16:40–53, 1999

Milan T, Verkasalo PK, Kaprio J, *et al*: Lifestyle differences in twin pairs discordant for basal cell carcinoma of the skin. Br J Dermatol 149:115–123, 2003

Montagna W, Carlisle K: The architecture of black and white facial skin. J Am Acad Dermatol 24:929–937, 1991

Neale MC, Boker SM, Xie G, *et al*: Mx: Statistical Modelling, 6th edn. Richmond, VA: Department of Psychiatry, 2002

Neale MC, Cardon LR: Methodology for Genetic Studies of Twins and Families, Vol. xxv. Dordrecht: Kluwer Academic Publishers, 1992, 496 pp (NATO ASI series. Series D, Behavioural and Social Sciences, Vol. 67).

Neale MC, Eaves LJ, Kendler KS: The power of the classical twin study to resolve variation in threshold traits. Behav Genet 24:239–258, 1994

Nurnberger F, Schober E, Marsch WC, *et al*: Actinic elastosis in black skin. A light- and electronmicroscopic study. Arch Dermatol Res 262:7–14, 1978

Olson RL, Gaylor J, Everett MA: Skin color, melanin, and erythema. Arch Dermatol 108:541–544, 1973

Pinnell SR: Cutaneous photodamage, oxidative stress, and topical antioxidant protection. J Am Acad Dermatol 48:1–19, 2003; quiz 20–12

Plomin R: Behavioral genetic methods. J Pers 54:226–261, 1986

Plomin R, DeFries JC, Loehlin JC: Genotype–environment interaction and correlation in the analysis of human behavior. Psychol Bull 84:309–322, 1977

Posthuma D, Beem AL, de Geus EJ, *et al*: Theory and practice in quantitative genetics. Twin Res 6:361–376, 2003

Reich T, Rice J, Cloninger CR, *et al*: The use of multiple thresholds and segregation analysis in analyzing the phenotypic heterogeneity of multifactorial traits. Ann Hum Genet 42:371–390, 1979

Sarkany I: A method for studying the microtopography of the skin. Br J Dermatol 74:254–259, 1962

Sarkany I, Caron GA: Microtopography of the human skin. Studies with metal-shadowed replicas from plastic impressions. J Anat 99:359–364, 1965

Scalia M, Geremia E, Corsaro C, *et al*: Lipid peroxidation in pigmented and unpigmented liver tissues: Protective role of melanin. Pigment Cell Res 3:115–119, 1990

Scarr S, McCartney K: How people make their own environments: A theory of genotype greater than environment effects. Child Dev 54:424–435, 1983

Seddon JM, Egan KM, Zhang Y, *et al*: Evaluation of skin microtopography as a measure of ultraviolet exposure. Invest Ophthalmol Vis Sci 33:1903–1908, 1992

Stamatas GN, Kollias N: Blood stasis contributions to the perception of skin pigmentation. J Biomed Opt 9:315–322, 2004

Sturm RA: Skin colour and skin cancer—MC1R, the genetic link. Melanoma Res 12:405–416, 2002

Sturm RA, Duffy DL, Box NF, *et al*: Genetic association and cellular function of MC1R variant alleles in human pigmentation. Ann NY Acad Sci 994:348–358, 2003

Taylor SC: Skin of color: Biology, structure, function, and implications for dermatologic disease. J Am Acad Dermatol 46:S41–S62, 2002

Thieden E, Philipsen PA, Heydenreich J, *et al*: UV radiation exposure related to age, sex, occupation, and sun behavior based on time-stamped personal dosimeter readings. Arch Dermatol 140:197–203, 2004

Toda K, Pathak MA, Parrish JA, *et al*: Alteration of racial differences in melanosome distribution in human epidermis after exposure to ultraviolet light. Nat New Biol 236:143–145, 1972

van der Mei IA, Ponsonby AL, Dwyer T, *et al*: Past exposure to sun, skin phenotype, and risk of multiple sclerosis: Case–control study. BMJ 327:316, 2003

Weigand DA, Haygood C, Gaylor JR: Cell layers and density of Negro and Caucasian stratum corneum. J Invest Dermatol 62:563–568, 1974

Whitfield JB, Cullen LM, Jazwinska EC, *et al*: Effects of HFE C282Y and H63D polymorphisms and polygenic background on iron stores in a large community sample of twins. Am J Hum Genet 66:1246–1258, 2000

Williams-Blangero S, Blangero J: Quantitative genetic analysis of skin reflectance: A multivariate approach. Hum Biol 64:35–49, 1992

Zhu G, Duffy DL, 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