Heritability and Gene–Environment Interactions for Melanocytic Nevus Density Examined in a U.K. Adolescent Twin Study

Rachel C. Wachsmuth,* Rupert M. Gaut,* Jennifer H. Barrett,* Catherine L. Saunders,* Juliette A. Randerson-Moor,* Ann Eldridge,† Nicholas G. Martin,† Timothy Bishop D,* and Julia A. Newton Bishop*‡

*ICRF Genetic Epidemiology Division, ICRF Clinical Center in Leeds, U.K.; †Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia; ‡ICRF Cancer Medicine Division, ICRF Clinical Center in Leeds, U.K.

Risk factors for melanoma include environmental (particularly ultraviolet exposure) and genetic factors. In rare families, susceptibility to melanoma is determined by high penetrance mutations in the genes *CDKN2A* or *CDK4*, with more common, less penetrant genes also postulated. A further, potent risk factor for melanoma is the presence of large numbers of melanocytic nevi so that genes controlling nevus phenotype could be such melanoma susceptibility genes. A large Australian study involving twins aged 12 y of predominantly U.K. ancestry showed strong evidence for genetic influence on nevus number and density. We carried out essentially the same study in the U.K. to gain insight into gene–environment interactions for nevi. One hundred and three monozygous (MZ) and 118 dizygous (DZ) twin pairs aged 10–18 y were examined in Yorkshire and Surrey, U.K. Nevus counts were, on average, higher in boys (mean = 98.6) than girls (83.8) ($p = 0.009$) and higher in Australia (110.4) than in the U.K. (79.2, adjusted to age 12 y, $p < 0.0001$), and nevus densities were higher on sun-exposed sites (92 per m^2) than sun-protected sites (58 per m^2) ($p < 0.0001$). Correlations in sex and age adjusted nevus density were higher in MZ pairs (0.94, 95%CI 0.92–0.96) than in DZ pairs (0.61, 95%CI 0.49–0.72), were notably similar to those of the Australian study (MZ = 0.94, DZ = 0.60), and were consistent with high heritability (65% in the U.K., 68% in Australia). We conclude that emergence of nevi in adolescents is under strong genetic control, whereas environmental exposures affect the mean number of nevi. *Key words: genes/heritability/melanoma/nevi/twins. J Invest Dermatol 117:348–352, 2001*

Exposure to sunlight is the most potent environmental risk factor for melanoma (Armstrong and Kricker, 1993). Evidence for a causal link is the overall gradation in incidence with latitude (Armstrong and Kricker, 1994). The presence of large numbers of nevi has also been shown to be a risk factor (Swerdlow *et al*, 1986; Augustsson *et al*, 1990; Bataille *et al*, 1996) and those living at lower latitudes may have larger numbers of nevi (Green *et al*, 1995; Sancho-Garnier *et al*, 1997). Therefore one hypothesis is that nevi are predominantly a marker of sun exposure (Armstrong and Kricker, 1996).

There is evidence, however, that nevi are under genetic control. Families prone to melanoma often have large numbers of nevi, or nevi of an atypical appearance (dysplastic) or distributed atypically (such as on the buttocks). This phenotype is usually referred to as the atypical mole syndrome (AMS), dysplastic nevus syndrome (Kraemer, 1987), or FAMMM syndrome phenotype (Bergman *et al*, 1992). Germline mutations associated with high penetrance have been identified in CDKN2A (Holland *et al*, 1995; Dracopoli and Fountain, 1996; Harland *et al*, 1997) and CDK4 (Zuo *et al*, 1996). Detailed phenotypic studies in U.K. families showed that whilst the atypical nevus phenotype correlated with mutation carrier status, implying that CDKN2A is nevogenic, the correlation was far from absolute (Wachsmuth *et al*, 1998; Newton Bishop *et al*, 2000). It is not yet clear whether the variation in expression of the phenotype within families is due to environmental or genetic modifiers.

Rare high penetrance mutations therefore exist that predispose to melanoma and, at least in part, expression of the nevus phenotype. It is our hypothesis that other variants controlling nevi expression may also be low penetrance melanoma susceptibility genes. The nevus phenotype is thus of interest as a marker of melanoma risk and in understanding the pathogenesis of melanoma.

We have undertaken a study of nevi in teenage twins to investigate the relative contribution of heredity and environment to nevus number. Heritability is defined as the proportion of variance explained by the additive effect of genes and thus depends on the prevalence of relevant genetic determinants in the population, the environmental exposures in that population, and the measuring instrument. This study is based on a previous Australian study (Zhu *et al*, 1999), in which 95% of the twins had U.K. ancestry but were

Manuscript received December 21, 2000; revised March 27, 2001; accepted for publication April 2, 2001.

Reprint requests to: Dr. Julia Newton Bishop, ICRF Genetic Epidemiology Division, ICRF Clinical Center in Leeds, St James's University Hospital, Beckett St, Leeds LS9 7TF, U.K. Email: j.newton-bishop@icrf.icnet.uk

Abbreviations: AMS, atypical mole syndrome; BSA, body surface area; DZ, dizygous; FAMMM, familial atypical mole and malignant melanoma syndrome; MZ, monozygous.

living with much higher levels of ultraviolet (UV) light. Evidence for the role of heredity on the nevus phenotype came from the Australian study, which estimated that 68% of the phenotypic variance was attributable to additive genetic factors. Because of the marked difference in environmental exposures in the two countries, heritability of nevus count in the U.K. would be expected to differ from heritability in Australia. By comparing our findings with those from this Australian study using the same definition of phenotype, we take advantage of the differences in UV exposures between the two continents in assessing the genetic and environmental determinants underlying nevi expression in adolescence.

In the general population melanocytic nevi start to develop from around 6 mo of life (MacKie *et al*, 1997). Peak numbers of nevi are seen around 40 y of age, and in very old people nevus numbers are small (MacKie *et al*, 1985). In biologic terms the process of maturation and disappearance of nevi may correspond to the cessation of proliferation of melanocytes and the induction of cellular senescence, and failure of this normal process may have a major role in the carcinogenic process for melanoma. It therefore seems likely that there are genetic and environmental factors that control the emergence of nevi and others that control the process of senescence of melanocytes. Adolescents were chosen in these twin studies as this is the time when nevi are increasing in number, and potential confounding factors leading to nevus senescence are unlikely to be operative. Whilst genes controlling senescence of nevi may be of great importance with regard to carcinogenesis, our main aim in this study was to consider genes controlling the emergence and proliferation of nevi.

MATERIALS AND METHODS

Recruitment of twins to the study One hundred and three MZ twin pairs and 118 DZ twin pairs between the ages of 10 and 18 y (inclusive) (mean age 14 y 4 mo) were included in the study. Twins were recruited via schools in the Yorkshire and Surrey regions of the U.K. between September 1997 and September 1998. Ethical committee approval was obtained for the study from a Multi-Regional Ethics Committee and all Local Ethics Committees. The schools were asked to distribute invitations to the twins attending their schools to take part in a study looking into ''genes and moles''. With the aid of cartoons the invitations described what the study involved and why the study was being undertaken. A dermatologist (RW) visited the twin pairs either at school or at home during daylight hours. In either location at least one parent was present. Following informed consent and during these visits, the twins had their nevi counted and answered individual questionnaires aimed at assessing UV exposure (to be reported elsewhere). The twins and their parent(s) were then asked to give either a 10 ml sample of blood (n = 690) or a 5 ml mouthwash with plain tap water for DNA analysis (n = 26).

Examination of the twins Benign melanocytic nevi were counted on 20 body sites, including the eye (iris and conjunctivae), buttocks, and anterior scalp, and were sized into <2 mm, 2–<5 mm, 5–<10 mm, and $\geqslant$ 10 mm categories using circular templates on acetate. Atypical moles were defined as having all three of the following characteristics: $\geqslant$ 5 mm in diameter, variable in pigmentation, and irregular in outline. Sun-protected sites were defined as upper arms, thighs, chest, abdomen, back, and buttocks, while sun-exposed sites were defined as face, neck, lower arms, lower legs, and dorsae of the hands and feet.

Height and weight were recorded for each individual and body surface area calculated as:

$$\text{BSA } (m^2) = [\text{height (cm)} \times \text{weight (kg)}/3600]^{0.5}.$$

The surface area of sun-protected *versus* sun-exposed skin was calculated using a modification of Wallace's rule of nines which takes account of age. Values appropriate to a child of 15 y old were used (McLatchie, 1990). The parts of the body were taken as representing units of the total body surface area as follows: face and neck (5.5%), lower arms and dorsae of the hands (9%), lower legs and dorsae of feet (16.5%), chest and abdomen (13%), back and buttocks (18%), upper arms (8%), and thighs (18%). Therefore the total percentage of BSA in sun-exposed sites was taken as 31%, and the total in sun-protected sites in which we counted nevi was 57%. Twelve percent of the surface area was not examined (posterior scalp, genitals, palms of the hands, and soles of the feet). Nevus counts were transformed into nevus densities by dividing the counts by the estimated body surface area (BSA) examined.

The same observer (RW) was used throughout this study in the U.K., to limit the effects of observer variability in counting. Unpublished data from other studies conducted by our Department suggest that inter-observer agreement in count is of the order of 80%, whereas agreement for counts on the same subject at different times by one observer is of the order of 96%. To facilitate the direct comparison with the Australian study, the key Australian research nurse, AE, visited Leeds to develop a unified nevus counting and scoring scheme with RW. This scheme was evaluated in the first 16 twin pairs of our sample with AE and RW each scoring both twins.

Zygosity determination Genomic DNA was extracted from blood and sputum samples using Nucleon BACC2 DNA extraction kits (Tepnel Life Sciences, Didsbury, U.K.) as per manufacturer guidelines or sent to Nucleon Biosciences (Tepnel Life Sciences) for extraction.

Zygosity determination involved genotyping by PCR a fluorescent multiplex consisting of six microsatellite markers. This multiplex, known as the Second Generation Multiplex (SGM) was obtained from the Forensic Science Service (Birmingham, U.K.). Reaction conditions are described elsewhere (Kimpton *et al*, 1996). A separate fluorescent reaction was also performed using the D9S942 microsatellite marker alone. All amplification reactions were carried out using Ampli*Taq*Gold polymerase (Applied Biosystems, Warrington, U.K.).

Electrophoresis was performed on an ABI377 automated DNA sequencer (Applied Biosystems). One microliter of denatured PCR product was loaded onto a 4.25% polyacrylamide gel (6 M urea; Anachem). Tamra GS500 (Applied Biosystems) was run in every lane as an internal size standard. Gel images were analyzed using Genescan 3.1 software (Applied Biosystems). Genotyping was performed using Genotyper 2.0 software (Applied Biosystems) to assign allele sizes in basepairs and also repeat numbers in the case of the SGM microsatellite markers.

Zygosity determination was performed on all same-sex twin pairs (166 pairs in total) using the seven microsatellite markers. 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.

Statistical analysis Analyses were performed using Stata Statistical Software (Version 6.0, 1999, Stata, College Station, Texas). Comparisons of means between the sexes and between sun-exposed and sun-protected sites were made using t tests. The relationship between nevus count and age was examined using linear regression. Comparisons were made both for absolute nevus counts (to allow for comparison with other reported studies) and for nevus density, as the twins were in a growth phase and included both sexes. Where nevi of a particular type were rare, i.e., 5 mm or more in diameter, or atypical, absolute counts were used rather than densities. Log transformations of nevus counts and densities were used in regression analyses and structural equation modelling to produce more normally distributed data. Log nevus counts were adjusted for age and sex. Comparisons of correlations in nevus count or density were performed using the Fisher transformation and assuming that the resulting statistic had a normal distribution.

Intra-class correlations were estimated using one-way analysis of variance. Structural equation modelling was used to estimate the additive genetic, common environmental and residual environmental components of variance under the assumption of no dominance variance. This was achieved by fitting an underlying model to the observed two-by-two covariance matrices for the MZ and DZ twins using the MX software (Neale *et al*, 1999). Each twin's phenotype (Y) is modelled as a linear combination of three standard normally distributed latent variables, representing additive genetic effects (A), environment shared between the twins (C), and individual environment effects and measurement error (E):

$$Y = aA + cC + eE.$$

As MZ twins share all their genes and DZ twins on average share half, and under the assumption that MZ and DZ twins share environmental effects with their cotwins to the same degree, the variance-covariance matrices can be predicted (Fisher, 1918). Under this model the variance for both MZ and DZ twins will be $a^2 + c^2 + e^2$, the covariance for MZ twins will be $a^2 + c^2$ and the covariance for DZ twins will be $a^2/2 + c^2$. The observed matrices were formed using all 206 MZ (or 236 DZ) twins to estimate the variances, because the order of twins within a pair is arbitrary (Sham, 1998).

RESULTS

Our sample of 221 twin pairs was found to consist of 63 female–female MZ, 40 male–male MZ, 25 female–female DZ, 38 male–male DZ, and 55 female–male DZ pairs (103 MZ pairs in total, 118 DZ pairs). The probability that same sex DZ twins were incorrectly classified as MZ was estimated to be less than 0.001 for each of 100 twin pairs and less than 0.01 for the remaining three twin pairs based on their genotypes and the allele frequencies of the markers in this population.

Table I shows the summary of the raw data. Boys had more nevi than girls, with a mean of 98.6 compared with 83.8 ($p = 0.009$). A mean of 46.9 nevi in boys and 38.3 in girls were 2 mm or more in diameter ($p = 0.01$). Ten percent of boys and 4% of girls had more than 100 nevi of 2 mm or greater in diameter ($p = 0.01$). Sex differences remained after subclassification of nevus size and after adjustment for BSA as shown in **Table I**.

Overall the nevus density in sun-exposed sites (mean 91.8, SD 56.5) was higher than in sun-protected sites (mean 58.1, SD 39.3, $p < 0.0001$). The difference between sun-exposed and sun-protected sites was greater for boys (mean density in sun exposed of 103 per m^2 *versus* 61.6 per m^2 in sun protected) than for girls (82.0 per m^2 *versus* 55.1 per m^2, $p < 0.0001$ for testing equal difference in boys and girls) (**Table I**). Furthermore, this difference was most marked for the smallest (< 2 mm) nevi in boys with an average density of 58.9 per m^2 in sun-exposed sites as compared with 28.8 per m^2 in sun-protected sites (**Table I**).

Table II shows the total nevus counts in boys and girls adjusted to age 12 y. The total number of moles was related to age in boys ($p = 0.002$) but not in girls ($p = 0.40$). In boys the number of moles increased on average by nearly 9% each year over this age range. There was no evidence of an increase in nevus density with age in either sex (data not shown).

The intraclass correlation for total nevus count and density in MZ pairs was 0.94 (95% CI 0.92–0.96) compared with 0.63 (0.52–0.74) and 0.61 (0.49–0.72), respectively, for the DZ pairs (**Table III**). The correlation estimates were higher for nevus density of nevi smaller than 5 mm in diameter than for numbers of large or atypical nevi. Interestingly, all correlations by nevus category and zygosity were greater for the sun-protected sites than for the sun-exposed sites but only the nevus count for nevi ⩾ 5 mm in dizygous twins approached significance when allowing for the number of comparisons made ($p = 0.02$ for the specific test of DZ twins with nevi ⩾ 5 mm).

Structural equation modelling gives estimates of the additive genetic component of variance (a^2) (**Table IV**). A strong effect of genes on nevus density, particularly of nevi smaller than 5 mm in diameter, was seen overall. The estimated heritability (a^2) was 65%, with an additional 29% of the variance attributable to shared environment (c^2), for "total nevus count". Results for total nevus density were similar to those for the counts. The genetic effect was higher in sun-exposed (78%) than sun-protected sites (51%), where the modelling suggests that there was a much greater effect (40%) of a shared common environment contributing to the high correlations observed between both types of twin.

The data on atypical nevi are difficult to interpret because of low numbers seen, but it is of note that the heritability of numbers of large and atypical nevi appears to be relatively low (16% in sun-exposed sites and 26% in sun-protected sites for atypical nevi).

Table I. Mean raw nevus counts and densities in boys and girls overall and in sun-exposed and sun-protected sites, with standard deviations (SD) and the statistical significance of the nevus phenotype by sex (p value)

	Boys (n = 211) mean (SD)	Girls (n = 231) mean (SD)	p value
Number nevi < 2 mm[a]	50.2 (26.9)	45.4 (29.7)	0.08
Nevus density < 2 mm[a]	39.5 (20.2)	35.3 (22.1)	0.04
Number nevi 2 to < 5 mm	44.1 (36.8)	36.6 (29.1)	0.02
Nevus density 2 to < 5 mm	33.9 (26.6)	28.2 (22.0)	0.01
Number nevi ⩾ 5 mm	2.8 (3.8)	1.7 (2.4)	<0.001
Number atypical nevi	1.2 (1.9)	0.8 (1.4)	0.01
Total nevus count[a]	98.6 (61.6)	83.8 (56.3)	0.009
Total nevus density[a]	76.7 (44.4)	64.8 (42.0)	0.005
Sun-exposed			
Nevus density < 2 mm[a]	58.9 (30.7)	48.8 (27.0)	<0.001
Nevus density 2 to < 5 mm	41.3 (36.6)	32.7 (26.7)	0.005
Number nevi ⩾ 5 mm	0.7 (1.4)	0.4 (0.8)	0.003
Number of atypical nevi	0.3 (0.7)	0.1 (0.4)	0.003
Total nevus density[a]	103.0 (61.9)	82.0 (49.3)	0.001
Sun-protected			
Nevus density < 2 mm[a]	28.8 (17.0)	27.9 (21.9)	0.62
Nevus density 2 to < 5 mm	29.4 (22.3)	25.6 (20.7)	0.06
Number nevi ⩾ 5 mm	2.0 (2.8)	1.3 (2.0)	0.007
Number of atypical nevi	0.9 (1.6)	0.7 (1.2)	0.08
Total nevus density[a]	61.6 (39.6)	55.1 (40.5)	0.09

[a]Nevi < 2 mm measured on 199 boys and 227 girls.

DISCUSSION

The comparison of nevus data between the U.K. and the Australian studies showed several remarkable and consistent features. While Australian adolescents (Zhu *et al*, 1999) had on average 30 more nevi than the British after adjusting for age (**Table II**, $p < 0.0001$), the measures of variation and covariation between individuals did not differ. This included the correlations in nevus density between MZ twins (0.94 in Australia, 0.94 in the U.K.) and DZ twins (0.60 in Australia, 0.61 in the U.K.) and the standard deviation of population nevus count (62.3 *vs* 60.2 for males, 56.4 *vs* 56.6 for females) (**Tables II, III**) (Zhu *et al*, 1999). Within each population, additive genetic factors were estimated to be the major source of interindividual variation in sex adjusted nevus density (an estimated 68% of variation explained by genetic factors in Australia and 65% in U.K.). The similarity in the estimated heritability from the U.K. and Australian studies together with our observation of higher heritability in sun-exposed (78%) *versus* sun-protected (51%) sites is suggestive of a threshold UV level for genetic expression that is attained in sun-exposed parts of the body even in the U.K. Conversely, common environmental factors account for a significant proportion (43%) of the variance in density of small nevi in

Table II. Comparison of mean (Mean) and standard deviation (SD) of total nevus counts in 12-y-old twins in the U.K. and Australia

	Males			Females		
	Mean	SD	n	Mean	SD	n
Total nevi adjusted to age 12 in U.K.[a]	88.1	60.2	199	71.4	56.6	227
Total nevi from Australian study	113.9	62.3	356	106.9	56.4	348

[a]To facilitate comparison between these two studies, the U.K. counts were adjusted to age 12 as this was the age of all the Australian twins.

sun-protected sites, perhaps due to higher interfamilial and individual variation in the exposure of so-called ''protected sites''.

In considering the raw nevus data we observed that there were more nevi among this U.K. group than expected and also that there was a relatively limited difference between the Australian and U.K. studies given the difference in UV exposure. In a 1992 U.K. study of 4–11 y olds, a mean of 31.3 nevi was counted (cf an estimated 79.2 in our study at age 12 y) (Pope *et al*, 1992). While the older age of the twins reported here would imply more nevi, the difference is surprising. These observations could imply that there is a cohort of young people in the U.K. who have had more sun exposure and are putatively at higher risk of melanoma, although it is not possible to exclude some effect of ascertainment bias in that the twins knew that we were interested in nevi. The same potential bias existed in Queensland, however, and comparison with recent population-based data there suggested little bias existed (Zhu *et al*, 1999).

Other characteristics of our population are in line with previously published work. We found in males a small increase in nevus number with age but not in nevus density, and males had significantly more nevi than females, as has been noted before (Pope *et al*, 1992; English and Armstrong, 1994; Green *et al*, 1995; Zhu *et al*, 1999). This could be either due to gender-specific differences in melanocyte biology or due to behavioral differences particularly relating to UV exposure between the sexes. We therefore looked at the gender differences in sun-exposed and sun-protected anatomical sites, which was most marked for smaller nevi (< 2 mm in diameter, $p < 0.001$, **Table I**). As we had also found a predominance of smaller (< 5 mm) nevi in sun-exposed sites suggestive of a greater UV effect on the development of smaller nevi, the data support the view that the higher nevus density in males is in large part explained by the greater number of small nevi in sun-exposed sites presumed to be due to greater recreational sun exposure early in childhood.

Numbers of atypical nevi showed relatively low correlation between twins, and the contribution of individual environment or measurement error was much greater than for other nevi (**Table IV**); however, as three-quarters had no more than one atypical nevus, the distributional assumptions behind structural equation modeling are not entirely met and the results should be viewed with caution.

The aim of this study was to quantify the degree to which genes and environment contribute to the variation in nevus number in adolescents in the general population. Performing essentially identical studies in two populations with markedly different levels of UV exposure but similar gene pools permits comparisons both within and between these populations. Furthermore, ensuring that

Table III. Intra-class correlations for (age and sex adjusted) log nevus densities or counts in MZ and DZ twins overall and in sun-exposed and sun-protected sites

Nevus size	MZ twins (103 pairs) Intra-class correlation (95% confidence interval)	DZ twins (118 pairs) Intra-class correlation (95% confidence interval)
Nevus density < 2 mm[a]	0.87 (0.83–0.92)	0.61 (0.50–0.73)
Nevus density 2 to < 5 mm	0.91 (0.88–0.95)	0.53 (0.40–0.66)
Nevus count ⩾ 5 mm[b]	0.52 (0.38–0.66)	0.32 (0.15–0.48)
Number atypical[b]	0.50 (0.36–0.65)	0.33 (0.17–0.50)
Total nevus count[a]	0.94 (0.92–0.96)	0.63 (0.52–0.74)
Total nevus density[a]	0.94 (0.92–0.96)	0.61 (0.49–0.72)
Sun-exposed		
Nevus density < 2 mm[a]	0.77 (0.68–0.85)	0.48 (0.34–0.62)
Nevus density 2 to < 5 mm[b]	0.84 (0.78–0.89)	0.42 (0.27–0.57)
Nevus count ⩾ 5 mm[b]	0.44 (0.29–0.60)	0.08 (0.00–0.26)
Number atypical[b]	0.27 (0.09–0.45)	0.17 (0.00–0.34)
Total nevus density[a]	0.88 (0.84–0.93)	0.50 (0.36–0.64)
Sun-protected		
Nevus density < 2 mm[a]	0.82 (0.75–0.88)	0.63 (0.51–0.74)
Nevus density 2 to < 5 mm	0.87 (0.83–0.92)	0.52 (0.38–0.65)
Nevus count ⩾ 5 mm[b]	0.48 (0.33–0.63)	0.36 (0.20–0.52)
Number atypical[b]	0.50 (0.35–0.64)	0.37 (0.22–0.53)
Total nevus density[a]	0.91 (0.88–0.94)	0.64 (0.53–0.75)

[a]Measured on 100 MZ pairs and 113 DZ pairs.
[b]Because of zero values, 1 was added to the count before taking logarithms.

Table IV. Variance components estimates of the best fitting model calculated using MX for log nevus densities or counts overall and in sun-exposed and sun-protected areas[c]

Nevus size	a^2 (95% CI)	c^2 (95% CI)	e^2 (95% CI)
Nevus density < 2 mm[a]	57.3 (38.1–81.7)	31.6 (7.3–50.4)	11.1 (8.1–15.5)
Nevus density 2 to < 5 mm	72.8 (51.6–92.9)	18.5 (0.0–39.9)	8.7 (6.4–11.9)
Nevus count ⩾ 5 mm[b]	50.4 (10.9–67.0)	5.4 (0.0–36.0)	44.2 (33.0–59.4)
Number atypical[b]	34.8 (0.0–62.7)	16.3 (0.0–47.0)	48.9 (37.0–64.3)
Total nevus count[a]	62.0 (44.4–85.4)	32.6 (9.1–50.5)	5.4 (3.9–7.5)
Total nevus density[a]	65.1 (46.6–89.5)	29.1 (4.5–47.8)	5.8 (4.2–8.1)
Sun-exposed			
Nevus density < 2 mm[a]	64.7 (38.3–84.0)	14.3 (0.0–38.4)	21.0 (15.5–29.0)
Nevus density 2–5 mm[b]	78.1 (51.3–87.2)	4.7 (0.0–30.5)	17.2 (12.8–23.5)
Nevus count ⩾ 5 mm[b]	40.3 (13.8–53.7)	0.0 (0.0–19.7)	59.7 (46.3–75.2)
Number atypical[b]	16.4 (0.0–41.2)	9.1 (0.0–33.1)	74.5 (58.8–90.7)
Total nevus density[a]	78.0 (54.7–91.7)	11.1 (0.0–34.3)	10.9 (8.0–15.1)
Sun-protected			
Nevus density < 2 mm[a]	39.8 (20.1–63.9)	43.2 (19.7–61.1)	17.0 (12.4–23.4)
Nevus density 2 to < 5 mm	67.5 (45.2–89.5)	19.8 (0.0–41.6)	12.7 (9.4–17.5)
Nevus count ⩾ 5 mm[b]	33.7 (0.0–63.3)	17.8 (0.0–47.3)	48.5 (36.2–64.8)
Number atypical[b]	26.4 (0.0–61.2)	24.2 (0.0–50.9)	49.5 (37.3–65.2)
Total nevus density[a]	50.7 (33.7–73.4)	40.2 (17.5–47.1)	9.0 (6.6–12.5)

[a]Measured on 100 MZ pairs and 113 DZ pairs.
[b]Because of zero values, 1 was added to the count before taking logarithms.
[c]The component of variance a^2 refers to the proportion due to additive genetic effects, c^2 to common environmental factors, and e^2 to residual environmental factors (see text for details).

the phenotypic measurements in the two studies were comparable allowed clearer interpretation of the results. The Queensland study provided strong evidence for the role of genetic factors but left open a number of hypotheses. We considered three separate hypotheses: (1) a simple model of gene–environment interaction, such that within each population environmental exposures affect almost uniformly each person's number of nevi where genetic factors are the major cause of variation between individuals (predicting observations essentially in line with those we observed); (2) an hypothesis that such genetic factors could only be revealed in the presence of high levels of UV exposure so that in the U.K. lower levels of UV might not be sufficient to express such strong genetic variation (which would be evidenced by a lower estimated additive genetic variance for the U.K.); and (3) the hypothesis that individuals would vary markedly in their response to UV exposure depending on their genetic make-up (which would be indicated by differing extent of variation in the number of nevi in the U.K. as compared with Australia). Whereas formal tests of such hypotheses are not possible, our data are clearly consistent with genes and environment acting separately and are inconsistent with the hypothesis that U.K. UV exposure fails to reach the appropriate critical level. Although the hypothesis of differential sensitivity to UV (in terms of nevus density) cannot be excluded, the similarities in the components of variation between the two studies suggest that such interactions are limited.

In summary, we have demonstrated a major effect of genes in controlling the emergence of nevi in adolescents. There was a demonstrable effect of sun exposure on the average expression of these genes, particularly nevi on sun-exposed body sites, but the major determinant of variation appears to be genetic.

This research was funded by the Imperial Cancer Research Fund and the NHS R&D Executive, Northern and Yorkshire Region. Our thanks to Mrs Bette Ward and Zoe Kennedy, for skilful assistance with administration and data entry for the study.

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