Genetics of Risk Factors for Melanoma: an Adult Twin Study of Nevi and Freckles

Veronique Bataille, Harold Snieder, Alex J. MacGregor, Peter Sasieni, Tim D. Spector

Background: We sought by use of an adult twin study to investigate the relative contribution of genetic and environmental effects on the expression of nevi and freckles, which are known risk factors for melanoma, and to determine if age and sun exposure influence the heritability of nevi. Design and Methods: Total nevus and freckle counts were conducted on 127 monozygotic twin pairs and 323 dizygotic twin pairs. Intraclass correlations were calculated by use of analysis of variance. Model-fitting analyses were performed to quantify the genetic and environmental components of the variance for nevus and freckle counts. Results: The intraclass correlation for total nevus counts was .83 in monozygotic pairs compared with .51 in dizygotic pairs. Quantitative genetic analyses showed that the contribution of genetic factors on nevi expression varied according to age. For twins less than 45 years old, the additive genetic variance on total nevus count was 36% (95% confidence interval [CI] = 0.8%–63%), with 38% (95% CI = 14%–61%) and 26% (95% CI = 16%–42%) of the remaining variance attributed to common environment and unique environmental effects, respectively. In twins aged 45 years or older, common environmental effects on total nevus count became negligible, with the additive genetic variance increasing to 84% (95% CI = 77%–88%). Body site was also found to affect the heritability estimates for nevus counts, with a statistically significant difference between sun-exposed and sun-protected sites. The polychoric correlation (i.e., the correlation in liability within twins for more than two categories) for total freckle counts was .91 in monozygotic twin pairs compared with .54 in dizygotic twin pairs. Additive genetic effects explained 91% (95% CI = 86%–94%) of the variance in freckle counts. Conclusion: The contribution of genetic factors on the variance for total nevus counts increased with age, and sun exposure appears to influence the expression of nevi. The results of this study highlight the need to take into account the age and site of nevus counts for future genetic linkage or association studies in the search for new melanoma genes.


Melanoma is now an important health issue in most Cauca- sian populations, since its incidence has risen steadily over more than 20 years (1). Melanoma can affect young individuals and has a very poor prognosis if not diagnosed early. Large epidemiologic studies, especially those looking at migrants to sunny climates, have clearly established that sun exposure and ethnic background are significant risk factors (2). However, the relationship between melanoma and sunlight is quite complex and does not seem to be dose dependent. Genetic factors are also important in melanoma, since chromosome losses are commonly found at multiple loci in melanoma tumors (3). Furthermore, germline mutations affecting a tumor suppressor gene, p16 or CDKN2, have been reported in up to 20% of the melanoma families worldwide (4–9).

Common and atypical nevi are the most powerful predictors of risk for melanoma in population-based as well as family studies (10–16). However, the predictive value of nevi as a marker of risk does vary with age, since nevi do show a statistically significant decrease with age, although it cannot be ruled out that this may be because of cohort effects (14,15). Individuals who belong to melanoma families often have large numbers of common and atypical nevi (17–19). This melanoma-associated phenotype is known as the atypical mole syndrome phenotype (AMS) and was first described simultaneously by Clark et al. (20) and Lynch et al. (21) in 1978. Most family studies that investigate potential linkage with the p16 tumor suppressor gene locus in melanoma families have concentrated on the melanoma phenotype. However, there is some recent evidence from two European melanoma family studies (22,23) that the p16 locus (CDKN2) may have a role in expression of nevi. Although most family studies have concentrated on studying the rare melanoma kindreds with multiple cases of melanoma, more power could potentially be derived from studying the “precursor phenotype” or the “intermediate phenotype.” Nevi are both precursors and markers for melanoma, since there is strong evidence that melanoma arises in pre-existing nevi, especially in patients with a genetic susceptibility to the disease (24). By studying nevi, one may be able to discover genes involved in the early steps of melanocytic differentiation. Studying a large, population-based cohort rather than rare families is more likely to yield results that are relevant to the whole population studied in terms of genes involved in nevus expression. Freckles are not precursor lesions for melanoma but are associated with a fair skin type that is a well-recognized risk factor for melanoma (25). Environmental factors, particularly sun exposure, are also involved in the expression of nevi. Comparisons between Aus-
tralia and the U.K. have shown, both in children and in adults, that high numbers of nevi are associated with high levels of sun exposure (26–28). However, whether this environmental effect only influences genetically susceptible individuals is not known.

Family studies investigate familial aggregation of disease but cannot easily distinguish between shared environmental and genetic factors. The twin model, by comparing similarities between identical and nonidentical (i.e., fraternal) twin pairs, is better suited to separate environmental and genetic influences on the liability to a trait or disease (29). This U.K. twin study is the first to investigate the relative contribution of genetic and environmental factors in the expression of nevi and freckles in adults through the study of twins.

**Subjects and Methods**

**Study Population**

The subjects consisted of 127 monozygotic twin pairs and 323 dizygotic twin pairs, all Caucasian and all female, aged 18 through 72 years, who were recruited from January 1997 through December 1997 from the St. Thomas U.K. Adult Twin Register in London. For historical reasons, the register consists mainly of female twins. Twins were not aware of the hypotheses being tested because they were part of a large study investigating many diseases and traits. Each underwent a detailed, nurse-administered questionnaire covering demographic information as well as days of exposure to natural and artificial sunlight throughout a lifetime. The data on sun and sunbed exposure will be part of a separate publication.

A standardized skin examination, including a full-body nevus count—divided into 17 body sites (excluding the genital area and posterior scalp)—was performed by nurses trained by one of us (V. Bataille) for 4 weeks before the start of the study. The nevus count protocol had been validated in two previous case-control studies of melanoma from our group (14, 15). All twin pairs were examined on the same day under the same circumstances in a well-lit examination room. Five research nurses performed the nevus counts, but each twin within a pair was always examined by the same nurse. Possible interobserver bias was assessed before the start of the study by having two different observers count nevi on 20 volunteers on two separate visits, yielding a correlation coefficient of .95 between the two examiners. Not all five nurses were assessed for interobserver bias. Nevi were recorded by size in three categories (≥2 mm and ≤5 mm, >5 mm and ≤10 mm, and >10 mm). The total-body nevus count was defined as the sum of all nevi greater than 2 mm in diameter. Large nevi were defined for the purpose of this study as melanocytic lesions of 5 mm in diameter or above (excluding congenital nevi). The counts of large nevi can be considered as a surrogate for atypical nevi, since most benign nevi at least 5 mm in diameter are atypical, with irregular edges and/or an irregular border. For the purpose of this study, nevi on sun-exposed sites were all nevi on the face, arms, and lower legs and nevi on sun-protected sites were nevi on the back, chest, abdomen, buttocks, and thighs. Skin type was assessed according to the classification of Fitzpatrick—type I: always burn and never tan; type II: often burn and tan lightly; type III: burn moderately and tan gradually; type IV: burn minimally and tan deeply. Hair and eye color was also recorded by use of the scoring by Gallagher et al. (30).

The mean age of the twins was 48 years (range, 19–73 years) for the monozygotic pairs and 44 years (range, 18–69 years) for the dizygotic pairs. Most twins were skin type II or III, and the distribution of skin types in monozygotic and dizygotic is shown in Table 1. The mean number of nevi was 35, with a median of 22 (range, 0–324). Fifty-five percent of the twins had fewer than 25 nevi, and 22% had more than 100 nevi (Table 1). The mean number of nevi decreased with age (P < .0001) (Fig. 1). The mean number of large nevi (melanocytic lesions with a diameter of at least 5 mm) was 1.4 (range, 0–88); 14% of the twins had more than two large nevi.

As expected, freckles were more common in twins with skin type I (P < .0001). Freckle counts were not affected by age. No freckles were more common in twins with skin type I (P < .0001). Freckle counts, >100 freckles, and common and large nevus prevalences in monozygotic and dizygotic twins* obtained with the statistical package Mx (33).

<table>
<thead>
<tr>
<th>Skin type</th>
<th>Monozygotic, No. (%)</th>
<th>Dizygotic, No. (%)</th>
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<tbody>
<tr>
<td>I</td>
<td>29 (12)</td>
<td>75 (12)</td>
</tr>
<tr>
<td>II</td>
<td>65 (28)</td>
<td>182 (30)</td>
</tr>
<tr>
<td>III</td>
<td>97 (40)</td>
<td>208 (34)</td>
</tr>
<tr>
<td>IV</td>
<td>49 (20)</td>
<td>146 (24)</td>
</tr>
<tr>
<td>Median total nevus count</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Median total freckle count</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>&gt;100 nevi</td>
<td>59 (23)</td>
<td>141 (22)</td>
</tr>
<tr>
<td>&gt;2 large nevi</td>
<td>39 (15)</td>
<td>89 (14)</td>
</tr>
<tr>
<td>Freckle counts, &gt;100</td>
<td>23 (11)</td>
<td>51 (11)</td>
</tr>
</tbody>
</table>

*Skin type was available in 240 M2 twins and 611 D2 twins.
A statistically significant association was found between freckle and nevus counts. Nevus numbers varied according to hair color: a median of nine for twins with red hair, 24 for twins with blond hair, 21 for twins with brown hair, and 14 for twins with black hair. However, this difference did not reach statistical significance ($P = .45$). The median number of nevi on the upper arms was four compared with two on the lower arm. Similarly for the thighs, the median nevus count was two compared with one for the lower leg. The median number of nevi on sun-exposed sites was seven compared with 19 on sun-protected sites. There was a highly statistically significant association between nevi on sun-exposed sites and nevi on sun-protected sites ($P < .0001$).

There was a highly statistically significant association between the number of common nevi and large nevi: 31% of the twins with 100 or more common nevi had two or more large nevi compared with 6% of the twins with 25 or fewer common nevi ($P < .0001$). Large nevi were more frequent in individuals with skin type II or III compared with individuals with skin type I or IV.

Heritability of Nevus Counts

The intraclass correlation coefficient for total nevus count was .83 in monozygotic twins compared with .51 in dizygotic twins. The effect of age was examined in more detail, separating the twins into two age groups (less than and at least 45 years of age) by use of the mean age as a cutoff point. Differences between intraclass correlation coefficients for total nevus count were higher for older twins than for younger twins: .67 and .58, respectively, in monozygotic and dizygotic below the age of 45 years and .86 and .42, respectively, in monozygotic and dizygotic pairs for twins aged 45 years or older (Fig. 2). The best-fitting model for total nevus count in the two age groups is...

Fig. 1. Nevus counts according to age groups, including all 450 twin pairs. The box and whiskers plots illustrate the median, interquartile range, and the range of the data. Outlying points are plotted separately.

Fig. 2. Correlations for total body nevus counts for twin 1 against twin 2 according to age groups and zygosity.
shown in Table 2. In twins aged less than 45 years, 36% of the variance in nevus counts was explained by additive genetic effects, with the remainder due to both unique and shared environmental effects. For twins 45 years old or older, the contribution of shared environmental variance was no longer statistically significant and could be set to zero (χ²[1] = .28; P = .60). As a result, the additive genetic variance was much greater in the older group (84%; 95% CI = 77%–88%) than in the younger group (36%; 95% CI = 0.8%–63%). Nevus numbers on sun-protected sites were more highly correlated within monozygotic and dizygotic pairs than for nevi on sun-exposed sites: intraclass correlations of .72 and .49, respectively, for sun-protected sites compared with .56 and .33 for sun-exposed sites. Estimates of variance components could not be set equal for sun-exposed and sun-protected sites (χ²[3] = 18.99; P <.0003). Table 3 shows estimates of the best-fitting model. The additive genetic variance for nevus counts on sun-exposed sites was smaller than that for nevus counts on sun-protected sites: 42% (95% CI = 17%–66%) versus 62% (95% CI = 44%–81%), respectively. Accordingly, both shared and unique environmental factors had a greater effect on the variance for nevus counts on sun-exposed sites.

Heritability of Skin Type and Freckle Count

Polychotic correlation coefficients for skin type were .76 in monozygotic twins compared with .38 in dizygotic twins. The best-fitting model included additive genetic effects (A = .78; 95% CI = 69%–85%) and unique environmental effects (E = .22%; 95% CI = 15%–31%). For total freckle count, the best-fitting model was an AE model with the lowest AIC, and 91% (95% CI = 86%–94%) of the variance was explained by additive genetic effects (Table 4). No statistically significant differences in the heritability of freckles was observed when analyzing body site of freckles separately.

Table 2. Variance component estimates of the best-fitting model for total nevus counts of twins aged less than 45 years and aged 45 years old or older*

<table>
<thead>
<tr>
<th></th>
<th>A (95% CI)</th>
<th>C (95% CI)</th>
<th>E (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twins aged &lt;45 y</td>
<td>.36 (.008–.63)</td>
<td>.38 (.14–.61)</td>
<td>.26 (.16–.42)</td>
</tr>
<tr>
<td>Twins aged ≥45 y</td>
<td>.84 (.77–.88)</td>
<td>—</td>
<td>.15 (.11–.21)</td>
</tr>
</tbody>
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*CI = 95% confidence interval; A = additive genetic variance component; C = common environmental variance component; E = unique environmental variance component. df = 5; χ² = 13.7; P = .06; Akaike’s information criteria = −0.25.

Table 3. Variance component estimates of the best-fitting model for nevus counts on sun-exposed sites and sun-protected sites*

<table>
<thead>
<tr>
<th></th>
<th>A (95% CI)</th>
<th>C (95% CI)</th>
<th>E (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun-exposed sites</td>
<td>.42 (.17–.66)</td>
<td>.24 (.04–.43)</td>
<td>.34 (.26–.44)</td>
</tr>
<tr>
<td>Sun-protected sites</td>
<td>.62 (.44–.81)</td>
<td>.19 (.02–.35)</td>
<td>.19 (.14–.25)</td>
</tr>
</tbody>
</table>

*CI = 95% confidence interval; A = additive genetic variance component; C = common environmental variance component; E = unique environmental variance component. df = 18; χ² = 19.4; P = .37; Akaike’s information criteria = −16.7.

knowledge, is the first to quantify in adults the relative contribution of genetic and environmental factors in the expression of these traits. Under the assumption that the shared environmental exposure is similar in monozygotic and dizygotic twin pairs, comparisons of monozygotic and dizygotic similarity allow for the estimate of the relative importance of genes and environment in the expression of a trait. This is possible because monozygotic twins share all of their genes, whereas dizygotic twins share around half of their segregating genes (29). Twin pairs have the advantage over sib pairs because they are precisely matched for age and cohort effect and there is no uncertainty about paternity. For nevus counts, which vary over a lifetime, accounting for the age effect is important. The first study ever to use the classical twin model to look at similarities between monozygotic and dizygotic twins was set up by Siemens (35) in 1924 and recorded nevus counts in young twins. Correlation coefficients for total nevus counts of .40 and .20 were reported for monozygotic and dizygotic twins, respectively. More recently, one small U.K. twin study (36) reported nevus numbers on 25 monozygotic and 25 dizygotic pairs. The correlation coefficients for nevus count in monozygotic twins were comparable to correlations reported in this study. However, for dizygotic twins, the correlations were lower than those reported here. The small sample size of the study makes the interpretation of the results difficult.

In this study, twins were Caucasian females recruited from all over the U.K. via the media. The twins were not aware of the hypotheses tested regarding skin phenotypes, since they were unselectively recruited. The mean number of common nevi recorded by trained research nurses was also very similar to another non-twin, U.K. population-based nevus count study performed by two dermatologists, so that the data can be generalized (15). Although intraobserver variability was not assessed in this study, it is unlikely that the small differences in nevus counts due to intraobserver bias had a substantial effect on the results, since these differences should have been similar for the monozygotic and dizygotic pairs and this study is comparing intrapair differences for monozygotic compared with dizygotic twins. Furthermore, the intraclass correlation coefficient for total nevus count in this study of .83 in monozygotic pairs can be regarded as a lower limit of reproducibility for nevus counts. Nevus count can be defined as a complex trait, since it is influenced by many factors and is extremely common in the normal population. Monozygotic and dizygotic pairs were similar regarding prevalence, mean number, and variance for nevus and freckle counts, which makes any unexpected biases in recruiting monozygotic compared with dizygotic twin pairs highly unlikely.
Quantitative genetic model fitting was used for this study, since estimates of genetic and environmental effects based on comparisons of intraclass correlations alone have relatively low power, large standard errors, and do not make full use of the information available in the variances and covariances (34). It involves solving a series of simultaneous structural equations to estimate genetic and environmental parameters that best fit the observed twin variances and covariances. In many instances, this model fitting has several advantages over the classic twin methodology, as seen in other twin studies (35). It allows for the separation of the observed phenotypic variance into its genetic and environmental components. However, large datasets are needed to confidently accept or reject a tested model, especially when attempting to quantify shared environmental factors. If we speculate that sun exposure is the most important environmental factor influencing nevi, the shared environment would be the sun exposure shared by both twins (such as family holidays), while the unique environment would be the sun exposure unique to one twin within a pair. For example, one twin may be a keen swimmer or keen tennis player, while the other may not be.

The variance in freckle count was almost entirely determined by genetic factors in this study, with no measurable common environmental effects. Freckles were counted on the face, back, and arms, and care was taken not to include solar lentigines on sun-exposed areas. The strong genetic influence on freckle counts may, in part, be explained by polymorphisms in the melanocortin 1 receptor (MC1R). In a twin study of children, an association study (37) has supported the role of MC1R polymorphisms in determining hair color and skin types. As expected, red hair and skin type I were strongly associated with freckle count in the study reported here. Furthermore, MC1R polymorphisms have been associated with melanoma susceptibility in sporadic and familial melanomas, and this is likely to reflect the increased risk of melanoma in fair-skinned individuals (38).

The higher degree of concordance in total nevus count in monozygotic compared with dizygotic twins confirms that the expression of nevi is statistically significantly influenced by genetic factors. The number of nevi decreases with age, as has been previously reported in the U.K. and Australia (14, 15). The greater additive genetic variance for total nevus counts in older twins, with a complete disappearance of common environmental factors, is likely to provide insight into the genetics of nevi over a lifetime. Older twins are likely to have a less-shared environment than younger twins because they are more likely to live apart, and the effects of education, diet, and upbringing may be less important with increasing age. Higher correlation coefficients for nevus counts with age may also be due to the influences of genes involved in the involution of nevi in older age groups. This disappearance of nevi with age may, for example, suggest the involvement of senescence genes. A possible candidate would be p16 (also known as CDKN2), a tumor suppressor gene associated with melanoma that has been shown to have an important role in cell senescence (39). Melanocytes in culture, taken from a melanoma-prone individual carrying a homozygous deletion of p16, appear to grow for more than 20 months, which is longer than ever reported for human melanocytes (Bennett D: personal communication). Furthermore, among melanoma families in the U.K., p16 mutation carriers have higher nevus counts than noncarriers (22). The CDKN2 locus has also been shown to contribute toward the expression of the nevus phenotype in Dutch melanoma families (23). However, segregation analyses of the nevus phenotype in French families with at least one case of melanoma suggest that the mode of inheritance of nevi is likely to be very complex (40).

A variety of genes may be involved in expression of nevi throughout a lifetime, but gene–environment interactions are likely to occur, since sun exposure is also important in the induction of nevi. Comparison studies (26–28) have shown that nevus numbers are greater in Australian children and adults that in age-matched individuals in the U.K. Nevi on sun-protected sites were under greater genetic influence than nevi on sun-exposed sites for twins of all ages in this study, which supports the role of sun exposure in the induction and involution of nevi. The fact that the upper arm had a higher median number of nevi than did the lower arm—with similar results for the legs—suggests that sun exposure may have a role in the involution of nevi on chronically sun-exposed sites. Indeed, data from our group, where a single examiner was involved in both studies, showed that the difference in nevus counts between Australia and the U.K. (i.e., with higher numbers of nevi in Australia in young age groups) is reversed in older age groups, with lower median nevus counts in Australians 45 years old or older compared with age-matched U.K. control subjects (28). This again implies that sun exposure may be involved in the involution of nevi in older age groups and that this response to sun with age may also be, in part, genetically determined.

The discovery of genes influencing expression of nevi is important and clinically relevant, since nevi are powerful predictors of risk for melanoma. Common and atypical nevi are not only markers of risk but, also unlike freckles, can be precursor lesions. Melanoma tumors may show a wide variety of chromosome losses, especially at the 9p21 chromosomal locus (CDKN2 locus), but germline mutations of p16 or CDKN2 are relatively rare, so that other melanoma genes remain to be discovered (41). Common regions of losses in melanoma tumors are chromosomes 1, 10, and 6—but none of these regions have, as yet, been shown to harbor a new melanoma tumor suppressor gene (42). Genetic changes in atypical nevi, studied by use of microsatellite markers and polymerase chain reaction analysis, are too rare to provide clues about early changes in precursor lesions (43). Furthermore, chromosome loss is unlikely to be the only mechanism leading to tumor initiation in melanoma, and other search strategies need to be implemented to discover melanoma genes or unravel the role of existing genes. Association studies using the intermediate or precursor phenotype in families as well as in population-based, case–control studies may, therefore, be very useful to discover genes involved in nevus expression.

This U.K. twin study confirms that genetic factors are very important in the expression of cutaneous traits associated with an increased melanoma risk. This study has shown that age has a significant effect on the heritability of nevi and highlights the need to carefully adjust or match for age in future genetic linkage or association studies involving nevus counts. Analyses of sun-exposed sites versus sun-protected sites confirm that environmental factors also play a substantial role in the expression of nevi and that the environmental influence on total nevus count varies statistically significantly over a lifetime. Future association or linkage studies by use of single or multiple cutaneous traits not only will shed some light on the role of existing candidate genes, such as p16, p19ARF, CDK4, and MC1R on nevus expression and skin type, but also may lead to the discovery of
new genes involved in skin pigmentation and melanocyte differentiation (44). With data on polymorphisms in known and novel genes, gene–environment interactions could also be investigated further. Moreover, once populations at risk of melanoma are characterized genetically, primary prevention of melanoma may be specifically targeted toward these “genotypically at risk” individuals.

APPENDIX

Fitting quantitative genetic models to twin data is based on the comparison of variance–covariance (or correlation) matrices in monozygotic and dizygotic twin pairs. The observed phenotypes are assumed to be linear functions of four underlying components of variance (45). Additive genetic variance (A) is the variance that results from the additive genetic effects of alleles at each contributing locus. Dominant genetic variance (D) is the variance that results from the nonadditive effects of two alleles at the same locus, summed over all loci that contribute to the variance of the trait. Shared or common environmental variance (C) is the variance that results from environmental events shared by both members of a twin pair. Specific or unique environmental variance (E) is the variance that results from environmental effects that are not shared by members of a twin pair and includes measurement error.

The genetic model can be represented by the following linear structural equations (32): 1) $P_i = aA_i + dD_i + cC_i + eE_i$ and 2) $V_P = a^2 + d^2 + c^2 + e^2$, where $P$ is the phenotype of the $i$th individual scaled as a deviation from zero. A, D, C, and E are coefficients of uncorrelated latent factors with zero mean and unit variance, while $a$, $c$, and $e$ are regression coefficients of the observed variable on the latent factors, inasmuch as they indicate the degree of relationship between the latent factors and the phenotype. $V_P$ is the phenotypic variance. Squaring the regression coefficients yields the (unstandardized) variance components ($V_A = a^2$, $V_D = d^2$, $V_C = c^2$, and $V_E = e^2$), whose sum is equal to the total phenotypic variance. Each of these components divided by the total variance $V_P$ yields the different standardized component of variance. Heritability can be defined as the ratio of additive genetic variance to the total phenotypic variance ($h^2 = V_A/V_P$). For monozygotic twins, correlations of the additive and dominance genetic factors between a twin and their co-twin are unity. For dizygotic twins, these values are .5 and .25, respectively. By definition, in both monozygotic and dizygotic same-sex pairs, correlations for common environmental factors are unity and for unique environmental factors are zero. The covariances in monozygotic and dizygotic twin pairs are thus equal to 1) $COV_{MZ}(P_1,P_2) = a^2 + d^2 + c^2$ and 2) $COV_{DZ}(P_1,P_2) = .5 a^2 + 0.25d^2 + c^2$.

The model further assumes random mating and absence of gene–environment correlation or interaction. In twin studies, the effects of D and C are confounded, which means that they cannot be included in the same univariate model. However, D and C have opposite effects on the patterns of monozygotic and dizygotic twin correlations. D tends to produce dizygotic correlations that are less than 50% of the monozygotic correlations, and C inflates the dizygotic correlations to be above 50% of the monozygotic correlations. Models constraining all genetic effects to be nonadditive are considered unlikely because they lack a plausible biologic interpretation.

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**NOTES**


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