A Population-Based Study of Australian Twins with Melanoma Suggests a Strong Genetic Contribution to Liability

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Melanoma runs within families, but this may be due to either shared genetic or shared environmental influences within those families. The concordance between pairs of non-identical twins compared to that between identical twins can be used to determine whether familial aggregation is due to genetic or environmental factors. Mandatory reporting of melanoma cases in the state of Queensland yielded approximately 12,000 cases between 1982 and 1990. Twins in this study and from the adjacent state of New South Wales (125 pairs in total) were used to partition variation in liability to melanoma into genetic and environmental factors. Identical twins were more concordant for melanoma (4 of 27 pairs) than non-identical twins (3 of 98 pairs; *P*-value $\approx$0.04). Identical co-twins of affected individuals were 9.8 times more likely to be affected than by chance. However, non-identical co-twins of affected individuals were only 1.8 times more likely to be affected than by chance. An MZ:DZ recurrence risk ratio of 5.6 suggests that some of the genetic influences on melanoma are due to epistatic (gene–gene) interactions. Using these data and population prevalences, it was estimated that 55% of the variation in liability to melanoma is due to genetic influences.

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INTRODUCTION

A number of investigations have examined the role of familial aggregation in cutaneous melanoma. Between 8 and 12% of melanoma cases are associated with a prior family history of the disease (Goldstein and Tucker, 1995). Duggleby *et al.* (1981) investigated the rate of melanoma in the relatives of 214 individuals with melanoma compared with that in the relatives of 194 age- and sex-matched controls. In the relatives of those with melanoma, there were 8 times more cases than expected from the contemporary prevalence rates for that region. Ford *et al.* (1995) showed that the risk of melanoma is 2.24-fold greater in those with a family history. However, Cockburn *et al.* (2001), like Nancarrow *et al.* (1993), point out that studies of nuclear families cannot distinguish between familial aggregation due to shared environment (such as similar patterns of sun exposure) and that due to shared genes.

The concordance between identical twins, who share all their genes, compared with non-identical twins, who share approximately half their genes, is an excellent basis on which to partition variation in a trait into genetic and environmental factors. Because identical twins control for genetic effects, it is possible to observe whether differential exposure to environmental factors in these twins concords with discordance for melanoma.

A genetic epidemiologic study of melanoma poses two main problems, regardless of whether we sample cases of twins and determine those with melanoma or vice versa. First, the incidence of melanoma is relatively low. Lichtenstein *et al.* (2000), in a study of 44,788 pairs of twins listed in the Swedish, Danish, and Finnish twin registries, found that only 290 twin pairs (107 identical, 183 non-identical) were discordant for melanoma and only 3 pairs (1 identical, 2 non-identical) were concordant for melanoma. A study by Milan *et al.* (2002) that used all the same-sex adult twins living in Finland on 31 December 1975 found that, of 12,941 twin pairs, there were 60 cases of melanoma, all from discordant pairs. In Queensland, Australia, the incidence was about 50 per 100,000 individuals in 1987 (MacLennan *et al.*, 1992) and about 69 per 100,000 individuals in 2002 (Coory *et al*, 2006). Therefore, samples from large catchments may not yield enough twins with melanoma to carry out meaningful

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Abbreviation: QFMP, Queensland Familial Melanoma Project

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analyses. Second, a protocol to ascertain twins affected with melanoma—or melanoma cases that are twins—may result in bias. For example, Mack *et al*. (2000) advertised in newspapers for twins with cancer or other chronic diseases and found that there were fewer disease-discordant non-identical twins and fewer older cases than expected. There may be some difficulty in ascertaining individuals who have already died from melanoma. Moreover, it has been noted that ascertainment through newspapers and popular media may be biased toward those who are more educated (Wainwright *et al*, 2005). This is relevant to this study as education may be associated with behavior related to sun exposure (Bastuji-Garin *et al*., 1999).

Fortunately, Australian law has enabled the collection of a data set that mitigates these two problems. In Australia, the law requires that the person in charge of a public or private hospital or a nursing home notify the cancer registry when a patient is known to be suffering from cancer. In practice, to capture all incident melanoma cases, this task is usually carried out by a variety of health services, particularly pathology services. The cancer registry for Queensland, the state in which this study was primarily conducted, began operating in 1982.

Using the data from the Queensland Cancer Registry, the Queensland Familial Melanoma Project (QFMP) further investigated families of cases diagnosed between 1982 and 1990. In the family (non-twin) data from this study, Do *et al*. (2004) investigated the genetics of age of onset of melanoma using the genetic relatedness between affected individuals and their parents, siblings, offspring, and other relatives. The results suggested that additive genetic and environmental factors were almost equal in importance to variation in age of onset. There was also evidence of non-additive genetic effects. It is interesting that environmental effects common to members of a family did not explain the aggregation of age of onset within those families. A hypothesis of Mendelian inheritance of a single major gene was rejected (Do *et al*., 2004).

With a population of approximately 2.5 million in the 1980s and a pair of twins for every 100 births, one would expect sufficient twin families within Queensland—where at least one twin individual in a pair has melanoma—to provide an adequate sample for genetic analysis. Using twins ascertained through the QFMP and NSW Central Cancer Registry, we carried out a classical twin analysis to determine the proportion of genetic and environmental factors influencing variation in liability to melanoma. We also investigated whether identical twins discordant for melanoma have a differential exposure to environmental factors increasing the risk of melanoma.

RESULTS

In total, our study identified 181 melanoma cases of twins from Queensland, between 1982 and 1990, and 144 from New South Wales, between 1985 and 1990. A calculation using the estimated population of Queensland, rates of twinning (Doherty and Lancaster, 1986), and rates of melanoma across this period (MacLennan *et al*., 1992) suggests that 181 twin individuals would be diagnosed with melanoma in Queensland during the period of this study. This provides confidence in the ascertainment procedure. Twin pairs were considered eligible if at least one twin individual was histologically confirmed with melanoma (primarily using pathology reports) and both twins in the pair survived to the age of 20. There were 121 eligible twin individuals from Queensland and 94 from New South Wales. Of these 215, there was no significant difference in the proportions of each sex (116 females, 99 males, $\chi^2_1 = 0.85$). However, either the proband or the co-twin in 49 pairs did not complete an interview, and a blood sample could not be obtained for 13 affected probands and 26 co-twins, leaving 127 twin pairs. Two probands (both from discordant pairs) were reviewed and reclassified as unaffected. Of the remaining twin pairs (125), there were 27 identical and 98 non-identical twin pairs (18 identical female, 9 identical male, 29 non-identical female, 25 non-identical male, and 44 non-identical opposite sex pairs). This ratio of $\sim$3 MZ:10 DZ (after accounting for ascertainment) is significantly different from the 1 MZ:2 DZ expected in European populations at the time of ascertainment ($\chi^2_1 = 8$, $P \approx 0.005$, Doherty and Lancaster, 1986). If the variation in liability to melanoma is due to genetic influences, we would expect a greater proportion of identical twins to concord for melanoma status, with both twins in a pair being either affected or unaffected, than non-identical twins. Consequently, we would expect fewer identical twins to be discordant for melanoma. Here, we ascertained cases in which at least one twin in a pair was diagnosed with melanoma. Hence, the observed ratio of MZ:DZ twins is consistent with the effect of ascertainment on familial cases of a strongly heritable disease. The average age at diagnosis was 45 years (Figure 1). Almost one-third of all the cases were diagnosed between the ages of 35 and 45.

There were four identical twin pairs and three non-identical twin pairs concordant for melanoma (Table 1). Fisher's Exact Test (Fisher, 1922) showed that the rate of concordance of melanoma for identical twins is significantly greater than that for non-identical twins (P-value ≈ 0.039, one-tailed). A small change in the number of concordant pairs may make this result insignificant at the 0.05 level.

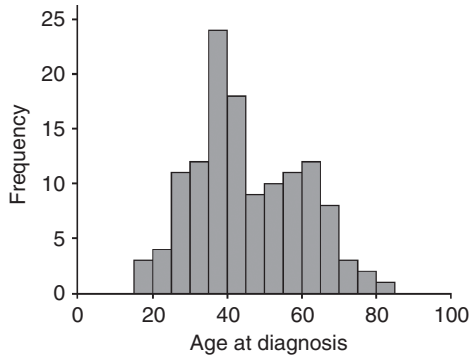

Figure 1. Age at diagnosis for affected twin individuals for whom information was available ($N = 128$).

Table 1. Twin pairs with complete information for melanoma status categorized according to concordance or discordance for melanoma and zygosity

Zygosity	Concordant	Discordant	Total	*r* (95% CI)
Identical (MZ)	4	23	27	0.55 (0.33–0.71)
Non-identical (DZ)	3	95	98	0.23 (0.03–0.40)
Total	7	118	125	

CI, confidence interval; DZ, dizygous; MZ, monozygous.
The tetrachoric correlation in liability to melanoma between twin pairs is based on the data presented and estimates of population prevalence.

Table 2. Contingency tables used to calculate tetrachoric correlations

	Affected	Unaffected
Identical twins		
Affected	4	36
Unaffected	35	13,258
Non-identical twins		
Affected	3	122
Unaffected	122	26,419

The numbers represent the total number of identical and non-identical twins pairs expected in the catchment area during the period when this study was conducted. These twins are categorized according to their observed and expected melanoma status.

In our sample, the unadjusted probability of developing melanoma if one's non-identical twin also has melanoma (i.e., recurrence risk) is about 6%. However, the probability of developing melanoma if one's identical twin has melanoma is 26%. Adjusting for year of birth, sex, and age (or age at diagnosis if the twin was affected), we expect 0.4 of the 27 identical co-twins of affected individuals to have melanoma; of the 98 non-identical twins of affected individuals, we expect that 1.7 individuals will be affected. From the numbers observed in this study, the chance of being affected with melanoma if one's identical co-twin is affected is 9.8 (approximate 95% CI: 0.41, 246.90) times higher (standardized risk ratio) than that for a person in the population with the same sex, age, and year of birth; the chance of being affected with melanoma if one's non-identical co-twin is affected is 1.76 (approximate 95% CI: 0.27, 11.34) times higher than that predicted by chance. This is an approximately 5.6-fold difference in the risk ratio between identical and non-identical twins.

Using these data, it is possible to estimate the relative effect of genetic and environmental influences on melanoma. Here, it is assumed that there is complete ascertainment of all twins within the population who have been affected with melanoma. This value is represented by the 325 twins ascertained (322 pairs). Twins for whom zygosity information was not available were allocated a zygosity group in the proportion of 3 MZ:10 DZ observed. Using this information, we have three of the four cells in the 2×2 contingency table. The missing cell is the number of twin pairs of whom both individuals are unaffected for melanoma, for both identical and non-identical twins. Assuming that we have captured all the concordant affected twin pairs, and using the expected rate of melanoma (MacLennan *et al*, 1992) and twinning (Doherty and Lancaster, 1986), we can determine the approximate number of twin pairs for the estimated population catchment. Then, noting that the approximate ratio of identical to non-identical twins is 1:2, we determine that the number of twin pairs in which both individuals are unaffected is 39,677 (13,258 identical twins and 26,419 non-identical twins; Table 2). A maximum-likelihood estimate of the tetrachoric correlation for the complete contingency table shows that the correlation between identical twins for liability to melanoma is 0.55 (95% CI: 0.33, 0.71) and that between non-identical twins is 0.23 (95% CI: 0.03, 0.40). Although confidence intervals are presented here, it should be noted that they are based largely on expected, but unobserved, data.

Using the differential in correlation between identical and non-identical twin pairs, we can estimate the proportion of genetic and environmental influences (Jinks and Fulker, 1970). Of the total variation, 45% is expected to be due to environmental influences unique to each twin. The remaining 55% is due to genetic influences. Of these genetic influences, two-thirds (37% of total variation) is due to additive genetic influences and one-third (18% of total variation) is due to non-additive genetic influences. Additive genetic influences are those that the effect on the trait is the sum of the allelic effects. Non-additive genetic effects include dominance and epistasis (interactions between genes at different loci). Environmental influences common to both twins in a pair were not a significant source of variation. The environmental influences found here have a differential effect between co-twins.

Age at diagnosis and histopathology for the seven pairs concordant for melanoma are shown in Table 3. In one pair of identical male twins, both individuals were diagnosed at the age of 36. One individual was affected with superficial spreading melanoma on the trunk, and the other was affected with melanoma, not otherwise specified, on the lower limb. A brother of these twins died at the age of 20 as a consequence of cutaneous melanoma on the lower limb (including the hip).

We have carried out several tests to exclude major biases in our study sample. The twin sample was collected within a registry-based study of melanoma that we believe gave complete coverage of all diagnosed cases within the study period. Tumor histological morphology and behavior did not differ between twin and non-twin cases (chi-square test, $P = 0.18$), or between monozygotic and dizygotic twins. The number of concordantly affected pairs was small, but a comparison of tumor histology did not suggest a large overrepresentation of milder cases (test for an excess of *in situ* lesions in non-proband twin cases, $P = 0.53$).

Table 3. Age at diagnosis and melanoma histology for the four identical and three non-identical twin pairs concordant for melanoma, without any particular order

	Twin 1		Twin 2	
	Histology	**Age**	**Histology**	**Age**
Identical	Superficial-spreading malignant melanoma	36	Malignant melanoma (not otherwise specified)	36
Identical	Superficial-spreading malignant melanoma	63	Malignant melanoma (not otherwise specified)	41
Identical	Malignant melanoma (not otherwise specified)	43	Superficial-spreading malignant melanoma	57
Identical	Superficial-spreading malignant melanoma	58	Melanoma *in situ*	62
Non-identical	Nodular melanoma	84	Melanoma *in situ*	78
Non-identical	Malignant melanoma (not otherwise specified)	24	Superficial-spreading malignant melanoma	41
Non-identical	Superficial-spreading malignant melanoma	72	Superficial-spreading melanoma *in situ*	57

Table 4. Identical and non-identical twin pairs discordant for melanoma categorized according to their comparative responses

	Identical twins (23 pairs)			Non-identical twins (95 pairs)		
	Affected	**Unaffected**	χ_1^2	**Affected**	**Unaffected**	χ_1^2
Who burned more easily?	0	0	—	15	7	2.9
Who tanned more easily (faster)?	0	0	—	6	16	4.5
Who usually had a darker tan?	0	0	—	10	17	1.8
Who spent more hours outdoors in summer						
At primary school						
On weekdays?	0	0	—	1	2	0.3
On weekends?	0	0	—	2	2	0
On summer holidays?	0	0	—	2	2	0
In your teens (13–19)						
On weekdays?	0	1	1	3	3	0
On weekends?	0	0	—	6	9	0.6
On summer holidays?	0	0	—	5	7	0.3
Who had more moles as a child?	0	0	—	0	1	1
Who had more moles as an adult?	1	1	0	5	2	1.3
Who had more sunburns as a child?	0	0	—	8	6	0.3
Who had more sunburns as an adult?	1	1	0	9	7	0.3
Who spent more time on the beach as a child?	0	0	—	1	2	0.3
Who spent more time on the beach as an adult?	4	0	4	14	12	0.2
Who tried more to get a tan as a child?	0	0	—	5	2	1.3
Who tried more to get a tan as an adult?	3	0	3	8	8	0

Only comparisons on which both twins agree are included in the counts. The χ^2 value is for McNemar's test. After correcting for the 17 tests, no result was significant at the 0.05 level.

Twin pairs discordant for melanoma are ideal for case–control studies to determine the effect of environmental and constitutive factors as they are matched for age and childhood environment and, in the case of identical twins, for genetics. From the results of the 17 comparative questions, Table 4 shows the number of pairs in the 2×2 table in which both twins agreed that one twin had a greater propensity or measure of one risk factor than the other twin. Many of the identical twins indicated that they were concordant for the risk factors or that they could not recall.

Nevertheless, four pairs of identical twins suggested that the twin affected with melanoma spent more time at the beach as an adult than his or her co-twin; no identical twin pairs recalled that the unaffected individual in a pair spent more time at the beach than his or her affected co-twin. McNemar's test showed that this difference was not significant at the 0.05 level after accounting for multiple testing (McNemar, 1947). For non-identical twins, 16 pairs indicated that the unaffected twin tanned more easily, whereas in 6 pairs the affected twin tanned faster. This difference was insignificant after accounting for multiple testing (Table 4). To further investigate the effect of sun exposure as an environmental risk factor, we compared the reports of sun exposure on weekdays in summer during adolescence for the affected twin with those of his or her unaffected co-twin (Table 5). A conditional logistic regression found that those unaffected with melanoma reported more time spent outdoors during this period than did the affected twins ($\chi^2_1 \approx 5.8$, $P \approx 0.016$). Further, we found that there was no significant difference in deterioration to the reticular patterning on the back of the hand, as measured on the Beagley–Gibson scale, between those affected with melanoma and those unaffected ($P \approx 0.64$, $N = 32$ twin pairs).

Table 5. Number of hours spent outdoors daily during summer between the ages of 13 and 19 comparing twins discordant for melanoma

	Unaffected			
	None	Up to 1 hr	1–3 hrs	More than 3 hrs
Affected				
None	0	0	1	0
Up to 1 hr	1	6	11	6
1–3 hrs	0	5	14	10
More than 3 hrs	0	4	1	8

A paired *t*-test was performed to determine whether there was a significant difference in mole count, freckling, or skin reflectance between individuals affected with melanoma and their unaffected co-twins. For pairs of non-identical twins discordant for melanoma, the affected individual had, on average, a greater number of moles on the face, neck, and torso, although this difference was not significant (Figures 2 and 3). A paired *t*-test showed that affected individuals had a greater number of moles on their arms and legs than their unaffected, non-identical co-twins (*P*-value ≈ 0.035) and their unaffected, identical co-twins (*P*-value ≈ 0.032). A plot of the number of moles on the arms and legs between twin pairs is shown in Figure 4. In pairs of non-identical twins, the affected twins had, on average, 80 moles on their arms and legs compared with 65 on their non-identical, unaffected co-twins. In pairs of identical twins, affected twins had, on average, 71 moles on their arms and legs compared with 46 on their identical, unaffected co-twins. Inspection of Figure 4 suggests that both twins in pairs in which both individuals were affected had similar numbers of moles on their arms and legs. The total mole count was greater for the affected individual in pairs of non-identical twins discordant for melanoma (*P*-value ≈ 0.047) but was not significantly different between pairs of identical twins discordant for melanoma (*P*-value ≈ 0.11).

A conditional logistic regression of pairs discordant for melanoma found that the odds ratio for each additional mole (on the arm or leg) against no moles to the onset of melanoma is 1.010 ($\chi^2_1 \approx 4$, 95% CI: 0.99, 1.021). There was no significant difference between identical and non-identical twins ($\chi^2_1 \approx 3.10$). Because melanoma is rare, this odds ratio is equivalent to the relative risk (Zhang and Yu, 1998). So, the risk of developing melanoma is 1.01^n, where n is the number of moles on the arms and legs. This risk is relative to someone with no moles. Therefore, those with 100 moles on their arms and legs are 2.7 times more likely to develop melanoma than those with no moles.

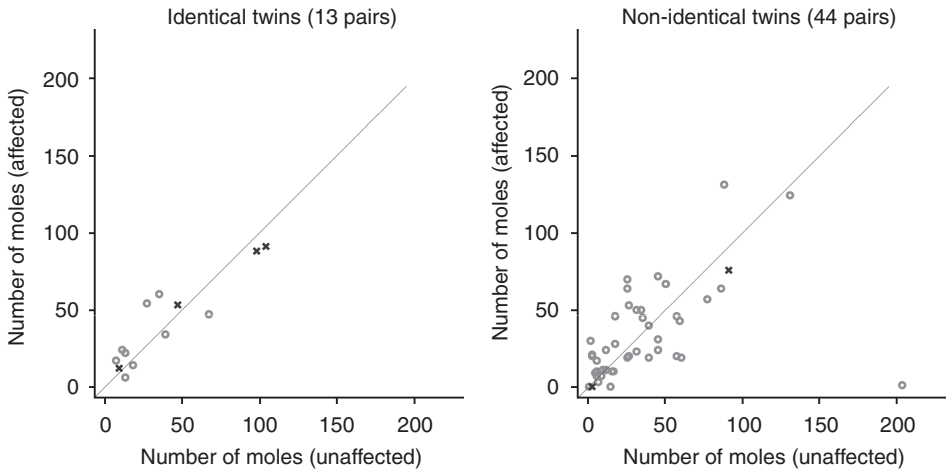

Figure 2. The number of moles counted on the torso comparing individuals affected by melanoma with their unaffected co-twin for identical (left) and non-identical (right) twins. Pairs of twins in whom both individuals are affected are presented (x) without any predefined order. Data on mole count were collected for only two of the three pairs of non-identical twins wherein both individuals were affected.

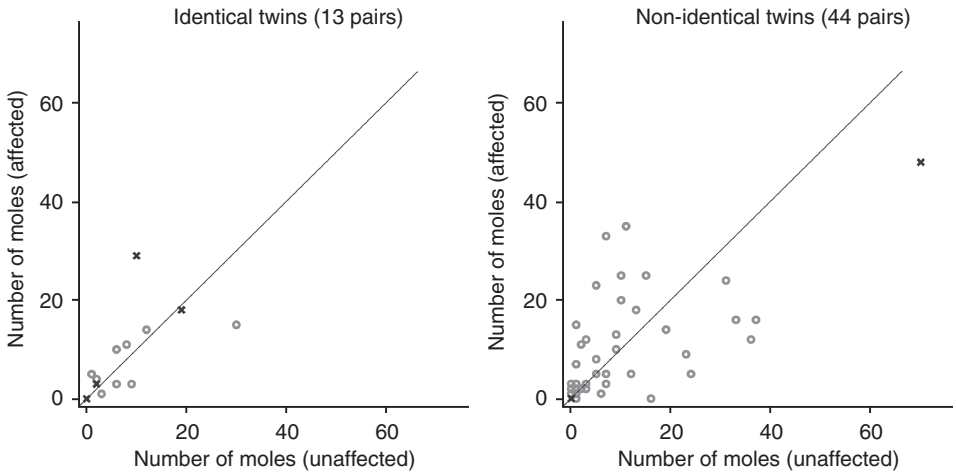

Figure 3. The number of moles counted on the face and neck comparing individuals affected by melanoma compared with their unaffected co-twin for identical (left) and non-identical (right) twins. Pairs of twins in whom both individuals are affected are presented (x) without any predefined order. Data on mole count were collected for only two of the three pairs of non-identical twins wherein both individuals were affected.

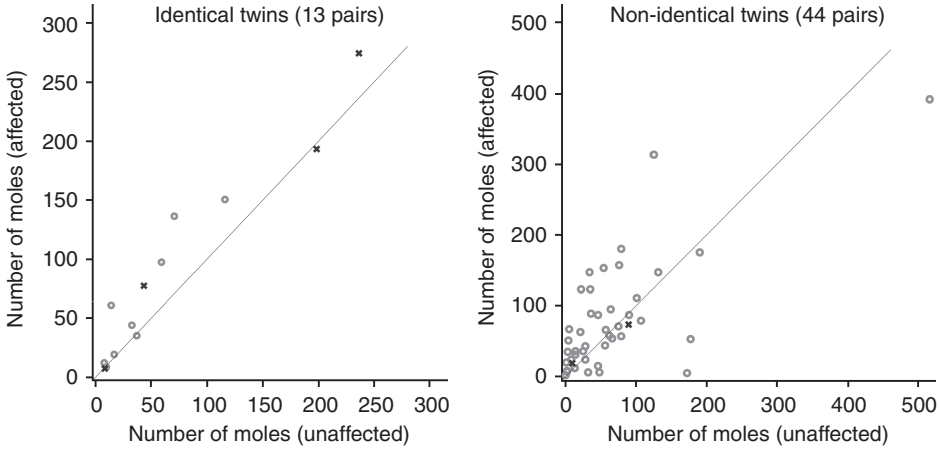

Figure 4. The number of moles counted on arms and legs comparing individuals affected by melanoma with their unaffected co-twin for identical (left) and non-identical (right) twins. Pairs of twins in whom both individuals are affected are presented (x) without any predefined order. Data on mole count were collected for only two of the three pairs of non-identical twins wherein both individuals were affected.

DISCUSSION

Lichtenstein *et al.* (2000), in a study on 44,788 twin pairs registered in Sweden, Denmark, and Finland, found 3 concordant pairs from 293 twin pairs in which at least one individual had melanoma. Perhaps because of the generally higher incidence of melanoma in Australia in general and in Queensland specifically, and the mandatory reporting of melanoma cases to cancer registries, we found 7 twin pairs concordant for melanoma from a total of 125 twin pairs. Although this number is small, it was sufficient to indicate that genetic influences were a significant source of variation in liability to melanoma. Although there are wide confidence intervals on the recurrence risks, identical twins were more than four times more likely to be affected with melanoma if they had an affected co-twin than non-identical twins, suggesting that non-additive genetic influences (dominance, epistasis) are a significant source of variation (Risch, 1990). This was echoed in the genetic modeling of variance in liability, in which the point estimate is that 18% of the variation in liability to melanoma is due to non-additive genetic influences. Do *et al.* (2004), who performed a genetic analysis of the non-twin data (from the larger study of which this is a part) for age of onset of melanoma, also found a point estimate suggesting non-additive genetic influence. Like Do *et al.* (2004), we found that variation in liability to melanoma was almost equally divided between genetic and environmental influences. Similarly, we also found that environmental influences common to twins in a pair were not a significant source of variation. This suggests that, for the most part, the aggregation of melanoma within families is due to genetic influences.

The second part of this investigation sought to determine whether there was a difference in environmental factors between those affected with melanoma and their unaffected

co-twins. Because identical twins control for genetic factors, discordance in melanoma status can arise only from the different environmental conditions experienced by the affected twin compared with those experienced by the unaffected twin, including stochastic events. Using data collected from questionnaires, there was a trend suggesting that those affected with melanoma had less routine time outdoors during adolescence and, subsequently, greater intermittent exposure to the sun compared with their unaffected co-twins. This accords with the meta-analysis performed by Gandini *et al*. (2005b) on the relationship between sun exposure and melanoma. However, it should be noted that public health campaigns outlining appropriate behavior in relation to the sun have been running in these catchment areas for some time (Coory *et al*, 2006). It is possible that the respondents' recall of events, after knowing that one twin individual has melanoma and understanding various risk factors, may also explain these results.

It is known that moles are a risk factor for melanoma (Gandini *et al*, 2005a). However, it is important to know the extent to which this risk can be attributed to genetic and environmental influences shared between mole count and melanoma. In this study, those affected with melanoma had a greater number of moles on their arms and legs than did their unaffected co-twins. This was the case for both identical and non-identical twins. Given that identical twins control for genetic influences, this suggests the importance of environmental factors that affect both moliness and risk of melanoma. Exposure to sunlight is the most obvious candidate (Gandini *et al*, 2005b). There was no significant difference in the number of moles on the torso or the head/neck between those affected with melanoma and their unaffected co-twins. However, there are generally few moles on the head/neck, and the torso is, by convention, generally covered.

Many studies have investigated the increase in the risk of developing melanoma with an increased number of nevi. Green and Swerdlow (1989) compared the relative risk of developing melanoma for nine case–control studies from various geographical locations. Although there were differences in the methods and sites of nevus count, the results suggest an appreciable increase in risk for each nevus. For example, Green *et al*. (1985) showed that the relative risk of developing melanoma in individuals with 2–4 nevi greater than 2 mm in diameter is 15.7 compared with those with no nevi. Holman and Armstrong (1984) found that those with 1–4 raised pigmented nevi had twice the risk of developing melanoma compared with those with no nevi. More recently, studies have shown that the relative risk for developing melanoma for those with more than 100 nevi compared with those with fewer than 10 nevi is about 10 (Garbe *et al*., 1994; Grulich *et al*., 1996). We looked at the effect of each additional mole on the arms and legs using twins discordant for melanoma as cases and controls. Naturally, they were matched for age and childhood environment. The increased risk of 1% for each additional mole (recurrence risk of 1.01) found here is smaller than that suggested by these previous studies. However, this risk appears broadly similar to that found using the 5135 non-twin individuals in the QFMP with information on nevi. Using these individuals, Aitken *et al*. (1994) found that the adjusted relative risk of developing melanoma for those with few nevi (compared with those with no nevi) is 1.9 and the relative risk for those with many nevi is 2.94.

Overall, these results confirm the heritability of risk toward developing cutaneous melanoma. They also show that the correlation between mole count and melanoma is not due purely to genetic effects common to both. Others have found that mutations in the *BRAF* gene are more common in melanomas occurring on intermittently sun-exposed skin (Pollock *et al*., 2003; Rivers, 2004). Hence, some of the variations in mole count may be a proxy for sun exposure and may directly increase the risk of melanoma.

MATERIALS AND METHODS

The data used in this study were collected as part of a larger study of individuals diagnosed with cutaneous melanoma between 1 January 1982 (coinciding with the beginning of the Queensland Cancer Registry) and 3 December 1990. The design and general characteristics of the participants of the QFMP have been outlined by Aitken *et al*. (1996) and Baxter *et al*. (2008). Briefly, of the 12,016 eligible cases, 8,412 returned a family history questionnaire and provided their written, informed consent; of these, 7,784 agreed to answer further questionnaires. This family history questionnaire included the question, ''Are you a twin?'' Those who answered ''yes'' were recruited for the study. To obtain additional twin pairs for this study, we also recruited melanoma cases from the adjacent state of New South Wales between January 1985 and December 1990. As far as possible, the protocol and questionnaires used in New South Wales were identical to those used in Queensland. For this study, twins were eligible if both individuals in a pair reached 20 years of age, when the cumulative risk of developing melanoma was thought to be appreciable. Further questionnaires were administered to affected twins and their co-twins regardless of whether those co-twins were affected. This investigation conformed to the principles set forth in the Declaration of Helsinki, and approval for the study was provided by the Human Research Ethics Committee at the Queensland Institute of Medical Research.

A follow-up questionnaire administered to the twins asked their primary place of residence for each decade of life; their outdoor behavior in relation to the sun; their hair, eye, and skin color; and the sensitivity of their skin to acute and chronic sun exposure. Each twin in a pair was asked (1) whether they, or their co-twin, had a greater propensity to burn and tan from sun exposure; (2) which twin had more moles; and (3) who had been exposed to more sunlight. Trained research nurses visited both twins and their co-twins, regardless of their melanoma status, and measured their age, their skin reflectance on the back of the left hand and the inner upper left arm, and the number of moles greater than 2 mm in diameter on the head/neck, torso, and arms/legs. In addition, an impression of the back of the left hand, while it was held in a loose-grip position, was taken using a quick-setting silicon solution to assess photoaging (Fritschi *et al*., 1995). Venous blood samples were also collected. A final questionnaire requested information about their birth and asked questions to elucidate whether the twins were identical or non-identical and whether they had been separated from their co-twin for

longer than a year. Zygosity was determined from the responses to questions regarding physical similarity and others' confusion in distinguishing between twins. Bonnelykke *et al*. (1989) show that the rate of misclassification using this method is approximately 4% (composed entirely of non-identical twins misclassified as identical). Martin and Martin (1975) found complete agreement between zygosity tested using blood samples and that assessed from a questionnaire for 47 twin pairs.

Some individuals in the QFMP were contacted again between 2002 and 2005, and a computer-assisted telephone interview was conducted. Further detail of this follow-up can be found in an article by Baxter *et al*. (2008). Of interest to this investigation is a telephone-interview question concerning the amount of time spent outdoors each weekday in summer between 9 AM and 5 PM from age 13 to 19 years. The possible responses were ''none,'' ''less than 1 hour,'' ''between 1 and 3 hours,'' and ''greater than 3 hours.'' For this question, information was collected from 67 complete twin pairs.

Analysis

Using these data, we performed analyses to determine whether identical twins are more concordant for melanoma status than non-identical twins. If identical twins were more concordant, it would suggest that there are genes influencing liability to melanoma. Using the known basis for similarity between monozygotic and dizygotic twins, correlations between twin pairs can be used to decompose the variance of a trait into genetic and environmental influences. Genetic variation can be partitioned into additive (A) and non-additive (D) influences. If the effect of an allele on a trait is independent of the other allele at that locus or of any other allele at any other loci, then additive genetic influences are the sum of the effects of all these independent alleles as they affect the trait (A). Identical twin pairs are perfectly correlated for additive genetic effects, whereas dizygotic twin pairs, who share roughly half their genes, are expected to correlate at about 0.5. Non-additive influences can be allelic interactions (dominance) or non-allelic interactions (epistasis). Allelic interactions are those that the effect of an allele at a particular locus is dependent on the other allele at that locus (Mendelian inheritance). Epistasis, also known as gene–gene interaction, is the condition in which the effect of an allele at a particular locus is dependent on an allele at another locus or on alleles at multiple loci. There is no possibility of distinguishing between dominance and epistasis in a classical twin design; hence, the non-additive term is usually called ''dominance,'' with dizygotic twins expected to correlate at 0.25 (Jinks and Fulker, 1970).

Variation due to the environment can be partitioned into either that influencing both twins (*C*) or that influencing each twin disproportionately (*E*). Twins are expected to correlate for common environmental factors (*c*) independent of whether they are identical or non-identical. By definition, twins are not correlated for unique environmental effects (Jinks and Fulker, 1970).

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. When the correlation between dizygotic twin pairs is less than half the correlation between monozygotic twin pairs, non-additive genetic effects have greater influence than common environmental effects. On the basis of a comparison of the monozygotic and dizygotic tetrachoric correlations, an ADE model was fitted to the data. Maximum-likelihood estimation of tetrachoric correlations and proportions of genetic and environmental influences on liability to melanoma was performed using the computer program Mx, version 1.54a (Neale *et al*., 2002). The analysis assumes a bivariate normal distribution underlying susceptibility to melanoma, with a threshold distinguishing those affected from those unaffected (Neale and Cardon, 1992).

Information on the cumulative risk of melanoma for each age, year of birth, and sex was obtained from the Australian Institute of Health and Welfare (http://www.aihw.gov.au/). These data were used to estimate the number of people whom we would expect to be diagnosed with melanoma had we selected individuals from the population with the same year of birth, sex, and age (or age at diagnosis if they were affected) as the co-twins of affected twins. This is calculated by summing the proportion of people affected for the cohort representing the year of birth, sex, and age at diagnosis (or current age if unaffected) for each co-twin of an affected individual. As the difference in incidence of melanoma within Queensland compared to that across Australia (Lens and Dawes, 2004) was relatively small considering the purpose of this study, the latter was used for greater accuracy.

In the second part of this study, we investigated whether exposure to different environmental factors is associated with discordance in melanoma status within a twin pair. In particular, we focused on identical twins because they control for genetic influences. The 2×2 contingency tables generated from questions requesting twins to compare their risk factors against those of their co-twins were analyzed using McNemar's test (McNemar, 1947). The *P*-value resulting from the chi-square test was adjusted for multiple testing using Bonferroni's correction.

Statistical tests were performed with SPSS (version 15) and R (R Core Development Team, 2008). In analyses in which co-twins were used as controls, the number of moles was transformed toward normality using $\log_{10}(x+1)$, where x is the number of moles, before carrying out paired *t*-tests. An analysis was performed to determine the increased risk of melanoma for each additional mole on an arm or a leg. This conditional logistic regression matched those with melanoma with their co-twins.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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