Genetic and Environmental Influences in the Development of Multiple Primary Melanoma

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Objectives: To identify risk factors and the prognosis associated with the development of multiple primary melanoma (MPM).

Design: Case-comparison studies of subjects with MPM and single primary melanoma. Sequencing of CDKN2A in germline DNA.


Patients: For mortality studies, 108 patients with MPM and 216 single melanoma controls matched for age, sex, site, and tumor thickness. For risk factor studies, 48 patients with MPM and 48 single melanoma controls matched as above. For CDKN2A analysis, a sample of 23 subjects with MPM.

Results: The development of MPM was found not to be an independent prognostic factor. The risk of MPM was greatest in those with a family history of melanoma, with large numbers of benign nevi, and the presence of clinically or histologically atypical nevi. Germline mutations of CDKN2A were present in 6 of 23 patients with MPM and in 5 cases consisted of the base pair substitution Met53Ile.

Conclusions: The importance of MPM should be addressed in melanoma follow-up protocols. Those patients at greatest risk can be identified by a family history of melanoma and their mole pattern. Germline mutations in CDKN2A occur in both familial and sporadic MPM and further studies are required to determine the value of analysis of this gene in melanoma surveillance. Patients should be informed that the development of MPM does not adversely affect their prognosis.

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PATIENTS with melanoma are at an increased risk of developing further primary melanomas. As the proportion of thin primary melanomas increases, so does the likelihood of developing a second primary melanoma relative to the risk of recurrence from the original primary. This clearly has implications for follow-up protocols. Although it might be expected that the development of a second primary melanoma would be associated with a worse prognosis, previous studies have not demonstrated this and the published literature suggests that individuals with multiple primary melanoma (MPM) may even have a better prognosis than those with a single primary tumor.

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For editorial comment see page 325

RESULTS

MORTALITY STUDY

Matching was satisfactory in that there was perfect matching by site and sex with no significant difference on average across the pairs for age and tumor thickness. We demonstrated no clear difference in sur-
PATIENTS AND METHODS

DATA COLLECTION

The Scottish Melanoma Group maintains a well-validated database that records clinical and pathological details of all invasive cutaneous melanomas diagnosed in Scotland since 1979. All patients registered with the Scottish Melanoma Group from the west of Scotland and Lothian regions between 1979 and 1996 with more than 1 histologically confirmed primary cutaneous melanoma were included in this study. Clark level 1 (in situ) melanomas were excluded since the biological significance of these lesions is not clearly established. All subjects gave informed consent and the study was approved by the local ethics committee.

One hundred eight patients with MPM were identified over the study period. They included 74 women and 34 men and the mean age at first melanoma was 52 years. Ninety-five individuals had 2 primary melanomas; 6, 3 melanomas; 2 each, 4, 3, and 6 melanomas; and 1, 8 melanomas. The first 2 melanomas were synchronous in 30 individuals; in those that were not synchronous, the median interval between the first 2 melanomas was 23 months (range, 2-180 months). The mean duration of follow-up from the first melanoma was 84 months (range, 0-250 months).

CASE-COMPARISON STUDY OF MORTALITY

A case-comparison study of melanoma-associated mortality was undertaken in which each of 108 patients with MPM was matched against 2 patients from the Scottish Melanoma Group database with single primary melanoma controlling for age, sex, tumor thickness, and body site of the first melanoma. The quality of matching was assessed by hypothesis tests and confidence intervals based on the binomial version of the McNemar test for binary matching variables and paired t tests for continuous matching variables.

Survival functions for single and multiple melanoma groups were independently estimated by the Kaplan-Meier method and compared using the log-rank test. To compare the survival of the 2 groups and incorporate the matching directly, 2 other methods were used. The first method was a direct application of McNemar test for paired data, where only the pairs with complete information are considered, ie, those pairs where at least 1 of the case and control pair have died of melanoma. Each multiple and single melanoma pair was categorized as to whether there was clear evidence of better survival in either the single or multiple melanoma or not. The second method used the paired Prentice-Wilcoxon test7 that takes the matching and censoring into consideration while comparing survival times for the single and multiple melanoma pairs.

Many factors are likely to influence survival for both multiple and single melanoma sufferers. Some of these were controlled for in the matching (age, sex, site, and tumor thickness) while others were not (eg, level of tumor invasion, ulceration of the tumor, and the individuals social deprivation category). Two distinct proportional hazards model approaches were used to compare the survivor functions of the 2 groups while controlling for any significant covariates. The first model ignored the paired structure of the data but included matched and unmatched covariates as potentially significant prognostic factors. The second model incorporated the paired structure of the data by introducing a parameter specifically intended to model the association between each member of a pair. Recent advances in modeling survival data where there may be an association between pair members have suggested using a gamma frailty proportional hazards model, which incorporates an unobserved random effect or “frailty” parameter shared by members within each pair. Any effect of covariates on survival is thus corrected for within-pair association.

CASE-COMPARISON STUDY OF RISK FACTORS

Forty-eight of the 108 patients with MPM were available for detailed questioning and examination. They were enrolled in a case-comparison study to investigate potential risk factors for MPM and details of 14 established melanoma risk factors investigated. A new control (single melanoma) patient was selected for each patient with MPM matched by age, sex, tumor thickness, and body site as mentioned earlier. Potential risk factors studied were tendency to sunburn, episodes of severe sunburn, ability to develop a tan, residence in a tropical or subtropical climate for more than 1 year, sunscreen use, eye color, natural hair color, freckling, family history of melanoma, family history of multiple nevi, more than 50 benign nevi, presence of clinically atypical nevi, histologically atypical nevi, and history of nonmelanoma skin cancer.

Point and confidence interval estimates of relative risk were based on the binomial version of McNemar test for each risk factor separately and stepwise conditional regression analysis correcting for all other possible risk factors. A classification tree to identify important interactions among potential risk factors was used while ignoring the matching structure of the data. The model was fitted by selecting the split at each level of the tree that best distinguished between multiple and single melanoma continuing until the data at each level were too sparse (n<20) or no further significant split in the data was possible. At each split the relative risk of a patient having a multiple melanoma rather than a single melanoma was estimated.

MOLECULAR GENETICS

DNA was extracted from peripheral blood of 23 patients with multiple melanoma involved in the case-control study of risk factors. Six of these had a confirmed family history of melanoma in a first-degree relative, the remaining 17 being sporadic. There was no known family history of pancreatic cancer. Exons 1, 2, and 3 of CDKN2A were amplified by polymerase chain reaction using previously published techniques. The polymerase chain reaction products were screened by single strand conformational polymorphism analysis following the method of Liu et al. Any variation in band shift from normal was followed by direct sequencing of the exon with the products analyzed on an ABI 373 DNA sequencer.
ness for ulcerated vs nonulcerated tumors and for men vs women (Table 1). However, there was no significant risk associated with tumor multiplicity using this model (P = .18) nor when a gamma frailty proportional hazards model was fitted (P = .15).

**RISK FACTORS**

All 14 potential risk factors for MPM were assessed individually and those identified to be significant are shown in Table 2. To identify independent significant risk factors for MPM correcting for all other possible risk factors a stepwise conditional logistic regression analysis was performed (Table 3). A classification tree was constructed to provide a better understanding of the importance of combinations and interactions of risk factors and their associated relative risk (Figure 2). Having a family history of melanoma carries a substantial risk of MPM.

![Figure 1. Mortality in multiple primary melanoma compared with matched single melanoma controls.](http://archderm.jamanetwork.com/pdfaccess.ashx?url=/data/journals/derm/11684/)

![Figure 2. Classification tree showing interactions of risk factors for multiple primary melanoma with associated relative risks. Risk is relative to a hypothetical subject at least risk (ie, sunscreen user with no family history of nevi or melanoma and < 50 benign nevi). The numbers within parentheses relate to the number of individuals at each node responding (yes) or (no) broken into those with multiple primary (given first) and single melanoma.](http://archderm.jamanetwork.com/pdfaccess.ashx?url=/data/journals/derm/11684/)

However, even in the absence of a family history of melanoma, a combination of a large number (ie, > 50) of benign nevi and not using a sunscreen involves a substantial relative risk (of the order of 100).

**MOLECULAR GENETICS**

Germline mutations in the coding regions of CDKN2A were detected in 4 of the 6 individuals with familial MPM and 2 of 17 cases of MPM as shown in Table 4. In each case the mutation was a single base pair (bp) substitution and in 5 consisted of the previously reported G-C substitution at codon 133 changing methionine to isoleucine at amino acid 53 (Met53Ile).\(^{12,13}\) The mutant protein has been shown not to bind CDK4 and CDK6.\(^ {13}\) The second mutation was a transition of glycine to thymine at base IVS 2+1. As the glycine is the first base of the second intron, this would be expected to affect gene splicing. Six of the remaining 15 sporadic patients with MPM had an identical polymorphism in exon 3. This was a glycine to cysteine transversion 29 bp downstream of the coding region.

The phenotype in the group with a mutation in CDKN2A was characterized by an earlier age at onset of the first melanoma (mean age, 37 years) than in the group without a mutation or with a polymorphism (mean age,
51 years). Cases 3 (familial) and 2 (sporadic) (Table 4) had an atypical mole pattern with more than 50 nevi, multiple clinically atypical nevi, and excised nevi showing atypical features. The other subjects had a normal mole pattern and none had developed nonmelanoma skin cancer.

### Table 4. Mutations in CDKN2A in Familial (f) and Sporadic (s) Multiple Primary Melanoma

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age at Onset, y/Sex</th>
<th>No. of Primaries</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Familial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f1 *</td>
<td>46/M</td>
<td>2</td>
<td>Met53Ile</td>
</tr>
<tr>
<td>f2 *</td>
<td>46/F</td>
<td>3</td>
<td>Met53Ile</td>
</tr>
<tr>
<td>f3</td>
<td>37/F</td>
<td>2</td>
<td>Met53Ile</td>
</tr>
<tr>
<td>f4</td>
<td>27/F</td>
<td>2</td>
<td>Met53Ile</td>
</tr>
<tr>
<td><strong>Sporadic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s1</td>
<td>35/M</td>
<td>8</td>
<td>Met53Ile</td>
</tr>
<tr>
<td>s2</td>
<td>29/F</td>
<td>2</td>
<td>G-T base 458</td>
</tr>
</tbody>
</table>

*Siblings.

In an original description of MPM in 1952 Pack et al observed that patients had a “surprisingly good end result of treatment.” Most subsequent studies have reported that the prognosis for patients with MPM appears no worse than for patients with a single melanoma. It has also been suggested that the prognosis may be better when compared with life tables or the population of patients with single melanoma from which the patients with MPM were drawn. In our previous description of the mortality from MPM in Scotland, we observed a lower mortality in a group of 38 patients with MPM compared with suitably matched controls with single melanoma. However, when the 2 groups were analyzed as 2 independent samples, the apparent survival advantage was not statistically significant (log-rank test, \( P = .06 \)). In this larger controlled study of 108 patients with MPM, we found no evidence that MPM is an independent prognostic factor. It is possible, however, that a study of this size has failed to detect a small effect on mortality. As the mortality in both cases and controls is generally low, the power to detect an effect is reduced. With the sample size, using the log-rank test has about 90% power to detect a 20% increase in 5-year survival for the multiple over single melanoma group.

Several risk factors for MPM have been suggested in uncontrolled reports. These include the Caledonian or “Celtic” phenotype (defined as sun-sensitive fair skin, red hair, and freckling), benign pigmented nevi, clinically atypical and histologically “dysplastic” nevi, nonmelanoma skin cancer, especially basal cell carcinoma, internal malignancy, and a family history of melanoma. The only previous case-control study of risk factors for MPM was from the Yale Melanoma Clinic in 1988. Eight cases of MPM and 24 controls with single melanoma (matched for age, sex, and survival) were studied excluding patients with a familial dysplastic nevus syndrome (defined in this study as the presence of dysplastic nevi and melanoma in 2 blood relatives). A significant association was found between MPM and the presence of 2 or more clinically atypical nevi (relative risk, 8.8) and histologically dysplastic nevi (relative risk, 6.2). These results are broadly similar to those reported with larger numbers of subjects in our present study. We found no evidence for an influence of the Caledonian phenotype, nonmelanoma skin cancer, or internal malignancy. However, patients with melanoma with large numbers of benign nevi or clinically atypical nevi, particularly if they failed to protect their skin from excessive sunlight, are at substantially increased risk of a second primary and should be kept under surveillance.

The strongest risk factor for the development of MPM in a patient with melanoma is the presence of melanoma in a first-degree relative. This effect is independent of the atypical mole syndrome phenotype. Although this may represent incomplete penetrance of a putative atypical nevus syndrome gene, other genetic mechanisms might operate. Mutations in CDKN2A are present in a variable proportion of familial melanoma reported from different geographical regions but are rarely present in sporadic melanoma. Of the 6 subjects with MPM and an affected first-degree relative in this Scottish population, 75% had a mutation in this gene. A high rate of mutation in CDKN2A has recently been reported from France in patients with MPM selected on the basis of familial melanoma.

Most cases of MPM do not occur in a family setting, 77% in this study. The only prior published report of molecular genetic analysis in this group comes from Monzon et al who demonstrated mutations of CDKN2A in 5 of 33 apparently sporadic cases of MPM, including the Met53Ile mutation that we have detected. On more detailed questioning of their subjects, 2 of the individuals with mutations were found to have a positive family history of melanoma leading the authors to suggest that a mutation in this gene may reflect an “occult” family history. The subjects in this study have been repeatedly questioned about a family history of melanoma. Where possible, the Scottish Melanoma Group database that records details of all cutaneous melanoma in Scotland since 1979 was cross checked for subjects with the same surname. We have confirmed that the 2 subjects, s1 and s2, with CDKN2A mutations are sporadic. We have yet to determine whether this is due to reduced or age-related penetrance, or a new mutation. In most cases sporadic melanoma without a mutation in CDKN2A, other genetic determinants may be important. However, the older age of this group suggests a larger influence of environmental factors.

In conclusion, we have demonstrated in a large cohort of patients with melanoma that the development of MPM is not an independent prognostic factor. Patients should therefore be treated and advised accordingly. While the CDKN2A gene is clearly important in familial MPM, the majority of MPM occurs in a family setting and is not related to a mutation of the CDKN2A gene.

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REFERENCES


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