EGF Gene Polymorphism and the Risk of Incident Primary Melanoma

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Abstract

Overexpression of the epidermal growth factor (EGF) pathway has been implicated in melanoma pathogenesis, and a recent case-control study identified a single nucleotide polymorphism (G to A) in the EGF gene where the G allele was associated with increased EGF expression and an increased risk of melanoma. To further evaluate this association, we conducted a case-control analysis from the Genes, Environment, and Melanoma study at the University of Michigan site using two different study designs. Incident cases of histopathologically confirmed first primary melanoma that were diagnosed between January 1, 2000 and December 31, 2000 from the University of Michigan Melanoma Clinic (n = 330) were compared with the following two different sources of nonmelanoma controls: spouse/friend controls (n = 84) and healthy volunteer controls from a case-control study of psoriasis (n = 148). Using a second analytic design, comparisons between multiple primary melanoma cases (n = 62) and single primary melanoma cases (n = 330) were also evaluated to estimate odds ratios (ORs). Genotyping for the single nucleotide substitution (G to A) at position 61 in the 5′ untranslated region of the EGF gene was performed from genomic DNA, and epidemiological risk factors were assessed through a telephone interview. When EGF genotypes were compared between incident primary melanoma cases and the nonmelanoma controls, the risk associated with the homozygous G/G genotype was not statistically significantly associated with an increased risk for incident primary melanoma compared with the homozygous A/A genotype [OR, 1.09; 95% confidence interval (CI); 0.65–1.85]. No strong associations with EGF G/G genotype were observed in comparisons of multiple primary and single primary melanoma cases (OR, 0.66; 95% CI; 0.25–1.73). Case subjects with tumors ≥3.5 mm compared with those <3.5 mm were not significantly associated with the G/G genotype (OR, 0.54; 95% CI; 0.12–2.35). Our data do not support a significant association between melanoma and the EGF 61*G allele or the homozygous G/G genotype. The EGF polymorphism is not a reproducible risk factor for melanoma or thick melanoma in our data. The two analytic approaches used in the study provide evidence against a strong association between EGF 61*G and melanoma and demonstrate the potential utility of case-control studies for evaluating the role of single nucleotide polymorphisms and cancer. Additional independent studies will be required to elucidate relationships between genetic variation in the EGF gene and risk of melanoma.

Introduction

Melanoma has increased substantially in incidence over the past half century to become one of the most common cancers in many populations of European origin. More than 54,000 new cases of invasive melanoma and 38,000 incident cases of in situ melanoma were be diagnosed in the United States in 2003. Invasive melanoma is expected to account for 1% of all cancer deaths in 2003. In patients with localized melanoma, tumor thickness (Breslow depth) is the most important prognostic feature. The 5-year survival rate for patients with T1 or thin tumors (<1.0 mm) is >90%, whereas for patients with T4 or thick tumors (>4.0 mm) with ulceration, the survival rate is 45% (2, 3).

Epidermal growth factor (EGF) is a member of the EGF superfamily, which also includes transforming growth factor-α, heparin-binding EGF-like growth factor, epiregulin, betacellulin, and amphiregulin (4). As a growth factor, EGF can activate DNA synthesis and cellular proliferation and stimulate mitogenesis in epidermal tissue (5, 6). In vitro studies have shown that growth factors produced by melanoma cells, including EGF, can have paracrine effects enhancing tumor growth (7). EGF is encoded by a 4.8-kb mRNA transcript from a gene that is 110 kb in length, contains 24 exons, and is located on human chromosome 4q25 (8). In a recent case-control study conducted in the United Kingdom, Shahbazi et al. (9) analyzed a region of the EGF gene from position −1350 to 164 and identified a G to A single nucleotide polymorphism at position 61 in the 5′ untranslated region, where the presence of the 61*G allele leads to increased in vitro EGF production in peripheral blood mononuclear cells in cell culture. Shahbazi et al. (9) then identified a significant association between homozygosity for the EGF 61*G allele and melanoma [odds ratio (OR), 4.9; 95% CI; 2.3–10.2]. Since the original report, a second recent case-control study conducted in the United Kingdom found no significant differences in EGF genotype frequencies among case and control subjects, although the frequency of the G/G genotype was over-represented among patients with thick tumors compared with thin tumors (10).

We conducted a case-control study from the Genes, Environment, and Melanoma (GEM) study at the University of Michigan site to (a) examine the original reported association between the EGF 61*G polymorphism and the risk for malignant melanoma using similar methods and two different study designs and (b) evaluate the hypothesis that EGF 61*G is associated with melanoma prognostic factors, i.e., Breslow depth.

Materials and Methods

Study Subjects. Study participants were recruited in an ongoing GEM case-control study at the University of Michigan. During a 12-month accrual period (January 1, 2000–December 31, 2000), all newly diagnosed cases of first primary melanoma at the University of Michigan were ascertained. Individuals with incident second (or higher order) melanoma were enrolled between January 1, 2000 and August 31, 2003. Nonmelanoma controls were obtained by recruiting the spouse or a friend of the case subject. Spouse/friend controls were eligible to participate in the study if they were not related to the case subject, did not have a history of melanoma, and lived within the same geographic region as the case subjects. Melanoma cases and their spouse/friend controls were recruited at the time of visit to the Multidisciplinary Melanoma Clinic at the University of Michigan Comprehensive Cancer Center (UMCCC) or through the Cancer Registry of the University of Michigan. Melanoma is the most common cancer diagnosed and treated at the UMCCC. In 2001, approx-
imately 65% of incident cases of melanoma diagnosed in the state of Michigan were seen at the UMCCC. The study-response rate among melanoma single primary case subjects was 78% and similar among the multiple primary cases (77%).

Study staff invited eligible subjects to participate, facilitated written informed consent, and obtained blood samples for DNA extraction. Interviewers called study subjects who had consented, and administered a standardized computer-aided telephone interview. Data collected included demographic information, individual and family history of melanoma, history of lifetime sun exposure (occupational and recreational), and other risk factors. Study subjects also completed a personal residence and work calendar, which collected the number of nevi and pigmented characteristic data, i.e., hair, skin, eye color.

Pathology slides were requested for all cases by the University of Michigan, retrieved by the center, and reviewed by a panel of dermatopathologists, who verified the validity of diagnoses as primary and invasive. Invasive melanomas were further categorized into histological types. Breslow depth and other pathology data were ascertained from and collected by the University of Michigan Cancer Registry for all melanoma cases. Breslow depth was categorized for statistical analyses as in situ, <1.5 mm, ≥1.5–3.49 mm, and ≥3.5 mm thickness.

An additional 148 unrelated, healthy individuals without psoriasis were recruited as control subjects for an allelic association study of psoriasis, an inflammatory hyperplastic skin disease with a strong genetic component (11). These controls were Caucasian volunteers who were recruited after responding to flyers posted around the University of Michigan Medical Center in the fall of 1997. This volunteer group was used as a second control group in the statistical analyses, and we were provided only with the DNA samples from them without personal identifiers, medical histories, or demographic characteristics.

Both the GEM and the Linkage Analysis of Familial Psoriasis studies were approved by the University of Michigan Institutional Review Board (IRB), and informed consent was obtained from all study subjects participating in both studies.

**Genotyping.** Genomic DNA from GEM study subjects was extracted from blood using the Puregene kit (Gentra Systems Inc, Minneapolis, MN). PCR amplification of a 242 bp region of EGF was performed in a 25-μl reaction containing 10 mm Tris-HCl (pH 8.3), 50 mm KCl, 2 mm MgCl₂, 0.2 mm each primer, 7 μM each primer (forward 5'-TGTCAGCTGAGAAAGGAGGT-3' and reverse 5'-TTACAGATTGATACGCC-3'), and 0.02 units of AmpliTaq Gold (Perkin-Elmer, Foster City, CA), and 40 ng of genomic DNA. PCR conditions were 95°C for 10 min followed by 35 cycles of 95°C for 30 s, 56°C for 1 min, and 72°C for 1 min followed by a final extension of 72°C for 10 min. Restriction fragment length polymorphism analysis was performed with the enzyme HhaI. The enzyme cut the 242 bp PCR product containing the 61*G allele into fragments 15, 34, and 193 bp in length. The 61*A allele introduced an additional restriction site after digestion with HhaI and cut the PCR product into 15, 34, 91, and 102 bp fragments. Digested products were separated on a 2.5% agarose gel and visualized by ethidium bromide staining. The restriction lengths of polymorphism analysis were confirmed by sequencing 12 study subjects for the G to A substitution at position 61 in the untranslated region of the EGF gene, with perfect correspondence.

Anonymized DNA samples from the Linkage Analysis of Familial Psoriasis study were prepared by the University of Michigan Institutional Review Board (IRB), and informed consent was obtained from all study subjects participating in both studies.

**Statistical Analyses.** Associations between melanoma risk and EGF genotype were evaluated using bivariate statistics for case-control studies as well as unconditional logistic regression methods to estimate ORs and 95% confidence intervals (CI) in multivariate analysis. Contingency tables were used to estimate ORs for the EGF genotypes and to calculate age- and sex-adjusted risks. The χ² test was used to estimate ORs and 95% CIs for the EGF genotypes taking advantage of this analytic design by using data from multiple primary melanoma cases.

The gender distribution was approximately equal among single primary incident case subjects; 52% (178 of 343) were male whereas 48% (165 of 343) were female. Approximately one-third of multiple primary cases were female (24 of 64). Spouse control subjects were more likely to be female (63%), because females were more likely to accompany spouses to clinic. The single primary case subjects were slightly younger at study enrollment than either the multiple primary cases or spouse/friend controls (52 years (SD ± 17), 57 years (SD ± 16), 55 years (SD ± 14)), respectively. Greater than 96% of study subjects reported Caucasian ethnicity (578 of 597). The remaining 4% of study subjects reported Southeast Asian (1 of 597), Middle Eastern (1 of 597), Indigenous American (2 of 597), Black African (1 of 597), and Other (14 of 597) ethnicities. Subsequent analyses were restricted to study subjects with self-reported Caucasian ethnicity including 330 single primary cases, 62 multiple primary cases, 102 incident in situ cases, and 84 spouse/friend controls. Among those single primary incident case subjects for whom we had uniformly reviewed melanoma histology data, 73% (230 of 314) of them had superficial spreading melanoma.

We calculated χ² statistics for testing the goodness of fit to determine whether the EGF allele frequencies were in Hardy-Weinberg equilibrium among the nonmelanoma spouse/friend controls (P = 0.7), volunteer controls (P = 0.2), and the control group reported previously by Shahbazi et al. (P = 0.7; Ref. 9). The calculated χ² values showed no evidence of deviation from Hardy-Weinberg equilibrium.

Table 1 shows the EGF genotypes and allele frequencies among first primary incident melanoma case subjects with nonmelanoma spouse/friend controls, volunteer controls, and the two control groups combined. Allele frequencies for 61*A and 61*G were similar among melanoma cases, spouse/friend controls, volunteer controls, and the combined control group. The homozygous G/G genotype was not statistically significantly associated with an increased risk for incident primary melanoma compared with the homozygous A/A genotype (OR, 1.14; 95% CI; 0.53–2.42) among single primary cases and the spouse/friend controls. After adjustment for age and sex, the G/G genotype point estimate did not change by >10% (OR = 1.21). Similarly, when EGF genotypes were compared between melanoma cases and the combined control set, the risk associated with the homozygous 61*G genotype compared with the homozygous 61*A genotype was not statistically significant (OR 1.09; 95% CI; 0.65–1.85).

Table 2 compares the EGF genotypes among multiple primary melanoma cases (n = 62) and single primary melanoma case subjects (n = 330). After adjustment for age and sex, the G/G genotype was not more common among study subjects with multiple primary melanomas compared with subjects with single primary melanomas (11.3 and 15.7%, respectively; OR = 0.66; 95% CI; 0.25–1.73).

Table 3 includes the distribution of EGF genotypes and allele frequencies by Breslow depth for 322 single primary incident melanomas, 54 multiple primary cases, and 102 case subjects with in situ melanoma. The frequency of the G/G genotype was lower among melanoma cases with tumors ≥3.5 mm (8.3%) compared with those <3.5 mm (14.4%). The G/G genotype was not statistically significantly associated with Breslow depth in melanoma cases with tumors ≥3.5 mm compared with those <3.5 mm (OR, 0.54; 95% CI; 0.12–2.35).
Risk estimates associated with self-reported pigmentation characteristics and family history of melanoma were also evaluated among single primary cases and spouse/friend controls (data not shown). The G/G genotype was not statistically significantly associated with hair, skin or eye color or a family history of melanoma in any relative or in first-degree relatives. Multivariate unconditional logistic regression was used to evaluate genotype as a risk factor for melanoma while adjusting for known risk factors: age at enrollment, sex, family history of melanoma in any relative, number of nevi, adult skin color, and hair and eye color. The point estimate for the G/G genotype adjusted for these potential confounders was not significantly different from 1.0 (OR, 1.61; 95% CI; 0.70–3.69).

We also compared the frequency of the EGF 61*G allele and EGF genotype in our two melanoma control groups to the original published United Kingdom series of controls and control subjects from a second report (9, 10). The frequency of the EGF 61*G allele among our spouse/friend and volunteer controls combined was lower than in the original published report, 38.4 versus 43.9% respectively (P = 0.18), but similar to the proportion reported in the second study, 38.4 versus 39.8%, respectively (P = 0.62). The combined control group G/G genotype frequency in the current study is also lower (12.9%) than in the original study (20.2%; P = 0.23) or in the second report (18.7%; P = 0.08). The frequency of the G/G genotype among patients with tumors at least 3.5 mm thick was lower in the current study (9%) compared with the original report (75%) or in the second report (30%).

**Discussion**

Analysis of 330 single primary melanoma cases, 62 multiple primary melanoma cases, 102 in situ cases, and 232 nonmelanoma controls from two different sources has allowed us to examine the original reported association between the EGF 61*G polymorphism and the risk for primary incident melanoma. Our data do not support the previously reported original finding of a significant association between the 61*G polymorphism and the risk of developing malignant melanoma (9). For those with the homozygous G/G genotype, only a 9% nonsignificant increase in risk for malignant melanoma was observed. Furthermore, there was no evidence that the G/G genotype was associated with Breslow depth.

Our results are consistent with a null association between EGF genotype and risk for malignant melanoma reported in a second study investigating EGF 61*G and melanoma susceptibility (10). Although we did not find a significant association between EGF genotype and disease progression, McCarron et al. (10) reported a modestly significant increase in the frequency of the G/G genotype among patients with tumor Breslow depth at least 3.5 mm compared with patients with tumors <3.5 mm. However, this association was based on a limited number of subjects with thick tumors.

### Table 1 EGF<sup>a</sup> genotypes and allele frequencies among first primary incident invasive melanoma case subjects and nonmelanoma spouse/friend controls, volunteer controls, and spouse/friend controls and volunteer controls combined

<table>
<thead>
<tr>
<th>Study subjects</th>
<th>Genotype</th>
<th>Number (%)</th>
<th>Odds ratio (95% CI)</th>
<th>Allele</th>
<th>Number (%)</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive melanoma cases (n = 330)</td>
<td>A/A</td>
<td>133 (40.3%)</td>
<td></td>
<td>A</td>
<td>411 (62.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>145 (43.9%)</td>
<td></td>
<td>G</td>
<td>249 (37.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>52 (15.8%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse/friend controls (n = 84)</td>
<td>A/A</td>
<td>32 (38.1%)</td>
<td>1.0</td>
<td>A</td>
<td>105 (62.5%)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>41 (48.8%)</td>
<td>0.85 (0.51–1.43)</td>
<td>G</td>
<td>63 (37.5%)</td>
<td>1.01 (0.71–1.43)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>11 (13.1%)</td>
<td>1.14 (0.53–2.42)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volunteer controls (n = 148)</td>
<td>A/A</td>
<td>52 (35.1%)</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>77 (52.0%)</td>
<td>0.73 (0.48–1.12)</td>
<td></td>
<td>181 (61.2%)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>19 (12.8%)</td>
<td>1.07 (0.58–1.98)</td>
<td></td>
<td>115 (38.8%)</td>
<td>0.95 (0.72–1.26)</td>
</tr>
<tr>
<td>Spouse/friend and volunteer controls (n = 232)</td>
<td>A/A</td>
<td>84 (36.2%)</td>
<td>1.0</td>
<td></td>
<td>286 (61.6%)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>G/A</td>
<td>118 (50.9%)</td>
<td>0.78 (0.54–1.12)</td>
<td></td>
<td>178 (38.4%)</td>
<td>0.97 (0.76–1.24)</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>30 (12.9%)</td>
<td>1.09 (0.65–1.85)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> EGF, epidermal growth factor; CI, confidence interval.

### Table 2 EGF<sup>a</sup> genotypes in case subjects with single primary and multiple primary melanoma

<table>
<thead>
<tr>
<th>EGF polymorphism</th>
<th>Single primary cases (n = 330)</th>
<th>Multiple primary cases (n = 62)</th>
<th>Adjusted odds ratio&lt;sup&gt;b&lt;/sup&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>133 (40.3%)</td>
<td>24 (38.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>G/A</td>
<td>145 (43.9%)</td>
<td>31 (50.0)</td>
<td>1.21 (0.67–2.20)</td>
</tr>
<tr>
<td>G/G</td>
<td>52 (15.7%)</td>
<td>7 (11.3)</td>
<td>0.66 (0.25–1.73)</td>
</tr>
</tbody>
</table>

<sup>a</sup> EGF, epidermal growth factor; CI, confidence interval.

<sup>b</sup> Adjusted for age and sex.

### Table 3 Distribution of EGF<sup>a</sup> genotypes and allele frequencies by Breslow depth among case subjects with in situ, single primary and multiple primary melanoma

<table>
<thead>
<tr>
<th>EGF polymorphism</th>
<th>Breslow depth in situ (n = 102)</th>
<th>0.1–1.49 mm (n = 300)</th>
<th>1.5–3.49 mm (n = 53)</th>
<th>≥3.5 mm (n = 23)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>29 (28.4%)</td>
<td>123 (41.0)</td>
<td>20 (37.7%)</td>
<td>9 (39.1%)</td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td>56 (54.9%)</td>
<td>132 (44.0)</td>
<td>24 (45.3%)</td>
<td>12 (52.2%)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>17 (16.7%)</td>
<td>45 (15.0)</td>
<td>9 (17.0%)</td>
<td>2 (8.7%)</td>
<td>0.54&lt;sup&gt;c&lt;/sup&gt; (0.12–2.35)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>114 (55.9%)</td>
<td>378 (63.0)</td>
<td>64 (60.4%)</td>
<td>30 (65.2%)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>90 (44.1%)</td>
<td>222 (37.0)</td>
<td>42 (39.6%)</td>
<td>16 (34.8%)</td>
<td>0.84&lt;sup&gt;c&lt;/sup&gt; (0.45–1.56)</td>
</tr>
</tbody>
</table>

<sup>a</sup> EGF, epidermal growth factor; CI, confidence interval.

<sup>b</sup> Adjusted for age and sex.

<sup>c</sup> P = 0.40 for difference between G/G genotype and A/A and A/G combined in tumors with a Breslow depth ≥3.5 mm vs. <3.5 mm.

<sup>d</sup> P = 0.58 for difference between G allele and A allele combined in tumors with a Breslow depth ≥3.5 mm vs. <3.5 mm.
The reasons for the differences in genotype frequencies among melanoma case subjects between our study and the original report are unclear. Shahbazi et al. (9) reported a higher G/G genotype frequency among malignant melanoma patients compared with the current study (47.4% versus 15.8%, respectively). Their case group included 81.2% invasive and 18.8% in situ malignant melanomas, and excluded patients with lentigo maligna and acral lentiginous melanoma. The University of Michigan GEM study included subjects with invasive single primary melanomas (79%) and in situ melanoma (21%). However, case subjects with in situ melanoma were included only in analysis of genotype and Breslow depth. In addition, the differences in genotype frequencies between the two studies are unlikely because of genotyping errors because we used the same restriction fragment length polymorphism methods and validated our results by sequencing analysis. Furthermore, we have shown that the allele frequencies are in Hardy-Weinberg equilibrium in the nonmelanoma spouse/friend controls and volunteer controls.

The reasons for the variation between our results and those of Shahbazi et al. (9) are unlikely because of survival bias. Only incident cases of melanoma were eligible for our study, whereas 43% of the malignant melanoma cases recruited by Shahbazi et al. (9) were prevalent cases. The median time from melanoma diagnosis to interview for cases in our study was 12 months. Furthermore, our response rate of 78% among single incident melanoma cases suggests that cases included in the present study are representative of all potentially eligible cases. Only 1.2% of the melanoma cases died before completing the interview. If survival of melanoma patients with the 61*G allele is improved relative to noncarriers, then using prevalent cases (those who survived longer) can potentially lead to a noncausal association between the 61*G allele and melanoma, because of the association between the 61*G allele and survival. However, we found suggestive evidence that 61*G homozygosity was somewhat less likely among advanced-stage melanomas compared with early-stage tumors. Therefore, it is unlikely that the 61*G allele is associated with improved survival in our sample. However, this conclusion is based on sparse data, because only two subjects were homozygous for the G/G genotype and had a Breslow depth of ≥3.5 mm.

Selection bias and/or population stratification may contribute to the discordance between the present study and the original report. The spouse/friend control G/G genotype frequency in the current study is slightly lower (13.1%) than in the previous study (20.2%). The frequency of the EGF 61*G allele among our combined control group is also lower compared with the original United Kingdom control series. These observations may reflect differences in the ethnic background of the study subjects used in the two studies. The control subjects selected by Shahbazi et al. (9) were a convenience sample described as “99 unrelated healthy white volunteers (mainly medical and laboratory staff)” whereas all control subjects in the current study self-reported Caucasian ethnicity. Without more detailed ethnicity information from the previous report, selection bias and population stratification cannot be excluded.

In the University of Michigan GEM study, the spouse control group could have potentially led to overmatching with respect to the exposures of interest. However, this is less of a concern in the present study of genetic factors because the main criterion for independent observations at a genetic level is lack of a blood relationship. In addition, we did not find a statistically significant association between EGF genotype and risk of melanoma among single primary cases and a second control set of volunteers. Furthermore, to validate our finding of no association between the EGF G/G genotype and the risk for incident primary melanoma, we used a second study design comparing “cases” with multiple primary melanoma to “controls” with first primary melanoma. The same source population of melanoma patients was used to identify both of these groups, and the absence of an association in this comparison provides further evidence that fails to support a strong association between EGF genotype and melanoma.

Incomplete control for confounding may have led to overestimation of the relative risk of the G/G genotype reported previously (9). Among Michigan GEM single primary case and spouse/friend control subjects, we were able to examine the association between EGF genotype and identified potential confounding factors: age at enrollment, sex, family history of melanoma in any relative, number of nevi, adult skin color, and hair and eye color. Adjusting for these factors had little effect on the relationship between EGF genotype and the risk of melanoma.

In our view, the most likely explanation of the discordance between the findings presented here and those by Shahbazi et al. (9) is related to sampling variation. Inherently there will always be random variation in epidemiological studies. Although our study included a larger number of cases (n = 330) and controls (n = 84 and n = 148 in two independent groups), additional independent studies will be required to elucidate any relationships between genetic variation in the EGF gene and risk of melanoma.

Melanoma produces many different growth factors (7). Increased levels of EGF and other growth factors may be the result of self-sufficiency in growth signals (an autocrine loop) produced by melanoma cells, especially in advanced melanomas. Using different immunohistochemical techniques, several studies provide evidence that the pattern of expression of receptors for EGF correlates positively with stage of development in melanocytic lesions (13, 14). Udart et al. (15) found that EGF receptor expression was much higher in melanoma metastases compared with superficial spreading melanomas, nodular malignant melanomas, and nevi and normal tissue. Furthermore, they observed a significantly higher copy number among melanoma metastases compared with primary melanomas. These results suggest that expression of EGF receptor may be increased in advanced stages of melanoma but not early lesions. Whether or not the EGF polymorphism is functional in vivo and influences gene expression during melanoma progressions requires confirmation, because the reported data demonstrating functional significance of this polymorphism was based on an assay of EGF production in peripheral blood mononuclear cells in cell culture.

This is the first study to take advantage of a novel study design comparing multiple primary cancer cases to single primary cases to estimate relative risk using an OR (12). Several assumptions are required to compare incident first primary melanoma cases to incident second (or higher order) primary melanoma cases. First, the second primary melanoma is independent of the first diagnosis. Second, the risk factor of interest (EGF genotype) is constant over time. Third, survival after diagnosis is unrelated to EGF genotype. These assumptions are likely to be met in the current study. Melanoma diagnoses were histologically confirmed as primary and invasive. Thus, misclassification of tumors as metastases is unlikely. The exposure of interest under investigation is a germ-line mutation, which is fixed within an individual and is in Hardy-Weinberg equilibrium within the study population. Lastly, the 61*G allele was not associated with improved survival in our sample.

Here we show that the results of our standard case-control analysis (using incident single primary melanoma cases and two different sets of controls) and the novel design (using multiple primary cases and single primary cancer controls) provide similar conclusions. The use of multiple control groups in the classic case-control design in conjunction with the use of two groups of melanoma cases to independently estimate ORs offers further evidence against a strong association of EGF variation and risk of melanoma.

Although the rational for studying EGF polymorphisms is biolog-
ically consistent in advanced melanoma, we could not find evidence to support an association between the EGF 61G>A polymorphism and susceptibility to incident melanoma. Other studies will be required to clarify any potential relationships between genetic variation in EGF and susceptibility to melanoma.

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EGF Gene Polymorphism and the Risk of Incident Primary Melanoma


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