Epidermal Growth Factor Gene (EGF) Polymorphism and Risk of Melanocytic Neoplasia

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A common single nucleotide polymorphism (SNP) in the 5’ untranslated region (5’UTR) of the epidermal growth factor (EGF) gene modulates the level of transcription of this gene and hence is associated with serum levels of EGF. This variant may be associated with melanoma risk, but conflicting findings have been reported. An Australian melanoma case–control sample was typed for the EGF+$61A>G$ transversion (rs4444903). The sample comprised 753 melanoma cases from 738 families stratified by family history of melanoma and 2387 controls from 645 unselected twin families. Ancestry of the cases and controls was recorded, and the twins had undergone skin examination to assess total body nevus count, degree of freckling and pigmentation phenotype. SNP genotyping was carried out via primer extension followed by matrix-assisted laser desorption time of flight (MALDI-TOF) mass spectroscopy. The EGF+$61SNP$ was not found to be significantly associated with melanoma status or with development of nevi or freckles. Among melanoma cases, however, G homozygotes had thicker tumors ($p = 0.05$), in keeping with two previous studies. The EGF polymorphism does not appear to predispose to melanoma or nevus development, but its significant association with tumor thickness implies that it may be a useful marker of prognosis.

Key words: epidermal growth factor/genetic variation/melanoma/nevi/predisposition


The mitogen-activated protein kinase (MAPK) pathway is a key signal transduction pathway in which extracellular growth factor signals are transmitted from cell surface receptors through a cascade of protein kinases to nuclear transcription factors, which regulate cell proliferation or differentiation by altering gene expression. Constitutive activation of this pathway frequently occurs in malignant melanoma and appears crucial for autonomous growth of these tumors (Cohen et al, 2002; Dong et al, 2003; Satyaamoorthy et al, 2003). Recently, somatic mutations of the BRAF gene have been shown to be the principal mechanism by which the MAPK pathway is activated in (20%–83%) melanomas (Brose et al, 2002; Davies et al, 2002; Dong et al, 2003; Maldonado et al, 2003; Pollock et al, 2003; Satyaamoorthy et al, 2003) and (74%–82%) benign melanocytic nevi (Dong et al, 2003; Pollock et al, 2003; Uribe et al, 2003; Yazdi et al, 2003). In the majority of melanomas without BRAF mutations, the MAPK pathway has been found to be activated through mutation of members of the RAS proto-oncogene family, particularly NRAS (van Elsas et al, 1996). Further support for this notion comes from the finding that we James et al, and others (Meyer et al, 2003), have shown that germline polymorphisms in the BRAF gene are significantly associated with melanoma predisposition. It is reasonable to expect that constitutive natural variation or somatic mutations in genes encoding other members of the MAPK pathway could confer inherited susceptibility to melanoma, or play a role in melanoma development.

The epidermal growth factor gene (EGF) encodes a ligand for the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase (RTK) that transduces growth signals to MAPK via RAS and BRAF. Shahbazi et al (2002) reported a striking association between melanoma risk and G/G genotype at the $+61A>G$ polymorphism in the 5’ untranslated region (5’UTR) of EGF ($p < 0.0001$). This was backed up by the finding that peripheral blood mononuclear cell cultures from individuals homozygous for the G allele produced significantly more EGF than cells from homozygotes for the A allele, while heterozygotes were found to produce intermediate levels (Shahbazi et al, 2002). The Breslow thickness of melanomas at time of presentation was also greater for G/G homozygotes ($p = 0.045$). A follow-up report failed to confirm the association with melanoma risk, although it did support ($p = 0.03$) an association between G/G genotype and thicker tumors (McCarron et al, 2003). In the present study, we sought to extend this work and to test the hypothesis that the nucleotide 61 polymorphism of the EGF gene predisposes to the development of nevi and freckles as well as melanoma.

Results

There were 3391 individuals genotyped where melanoma status was also known. Case and control genotype

Abbreviations: EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; SNP, single nucleotide polymorphism
frequencies were in Hardy–Weinberg equilibrium. Allele and genotype frequencies did not differ significantly between melanoma cases and controls (Table I). Comparing our sample to those previously studied found that all the control genotype frequencies could be equated (p = 0.133). The case genotype frequencies observed in our Australian sample did not differ significantly from those in a UK sample described by McCarron et al (2003) (homogeneity of genotype frequencies p = 0.25; homogeneity of association with melanoma, p = 0.09), but were significantly different from that in the UK cases described by Shahbazi et al (2002) (overall homogeneity p = 0.00001).

We did detect a weak association between genotype and tumor thickness (Table II; Kruskal–Wallis p = 0.049), which was similar in magnitude and direction to that found in both previous studies. The effect is small: only a fall from 21.4% in situ melanomas in the A carriers to 16.8% in the G homozygotes. Summarizing the data from the present study using the same four thickness categories as the two previous studies led to a small loss of information: the ordinal logistic regression model obtained p = 0.06. Combining the present study with the previous two found no evidence for heterogeneity of association, with the overall association p = 0.02.

Within the control twin families, there was no association between EGF + 61 genotype and total body nevus count (geometric mean count A/A, 98.6; A/G, 100.8; G/G 101.9; p = 0.56). This was also the case for subtotals of papular, macular or atypical nevi and for freckling score (data not shown).

We did not see the extreme association with melanoma risk observed by Shahbazi and coworkers (2002), but our findings are very close to the study of McCarron et al (2003). It is difficult to imagine ethnic differences leading to such a large discrepancy between study findings among cases only. As noted earlier, control genotype frequencies were very comparable across all three studies, so one plausible explanation is Type I error in the initial publication.

There are several lines of evidence that make an association between EGF genotype and melanoma behavior plausible, in keeping with the functional results presented by Shahbazi et al (2002). As noted earlier, BRAF somatic mutations are extremely common in cutaneous melanomas and benign melanocytic nevi, and, along with NRAS mutation, lead to the constitutive activation of the MAPK pathway that appears crucial to autonomous growth of these tumors. Animal models further implicate this pathway in melanocytic and melanoma pathogenesis. The Tu melanoma locus in Xiphophorus is known to be Xmrk, an RTK homolog of the EGFR (e.g. Wittbrodt et al, 1992; Winkler et al, 1994). Overexpression of this gene in medaka fish along with injection of TGFα leads to a similar phenotype. Similarly, Sutton et al (2002) have demonstrated that TGFα overexpression in mice leads to ocular melanocytosis.

We conclude that EGF polymorphism does not appear to predispose to melanoma or nevus development, but its significant association with tumor thickness implies that it may modify tumor progression.

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<tr>
<th>Table I. CMM and EGF + 61 genotype in three studies</th>
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<td>Controls</td>
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<td>A/G</td>
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<td>G/G</td>
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<td>HWE-P</td>
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<td>p-value</td>
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*CMM, cutaneous malignant melanoma. Count (percent) of each genotype. HWE-P represents p-value from exact test for Hardy–Weinberg equilibrium. Assoc LOD represents the contingency χ² for each 2 × 3 table converted into a LOD score (via inversion of the p-value).

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<th>Table II. Breslow thickness of Queensland melanomas versus EGF + 61 genotype</th>
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<td>Breslow thickness</td>
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<td></td>
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<tr>
<td>In situ</td>
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<tr>
<td>0.1–1.5 mm</td>
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<td>1.5–3.5 mm</td>
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<td>&gt;3.5 mm</td>
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Count (percent) of each genotype. EGF, epidermal growth factor.
Participants An Australian case–control panel was made up of 753 melanoma cases from 738 families participating in the Queensland Familial Melanoma Project (QFMP) (Aitken et al, 1994), and 2387 controls from 645 twin families enrolled in the Brisbane Twin Nevus Study (BTNS) (Zhu et al, 1999). Ancestry of the cases and controls was recorded (grandparental country of birth and ethnicity), along with phenotypic risk factors such as hair and eye color, and tanning type. Tumor thickness and level were recorded for the cases.

The BTNS twins and their siblings closest in age have all undergone total body skin examination at age 12 years by a trained nurse who assessed nevus count, degree of freckling and pigmented phenotypes (Zhu et al, 1999). Parents of the twins were genotyped, but only self-reported pigmented characteristics are available for them. No members of the BTNS have been diagnosed with melanoma. Approval to undertake this study was granted by the Human Research Ethics Committee of the Queensland Institute of Medical Research, and all participants gave their signed informed consent.

Genotyping The functional EGF promoter SNP (rs4444903) was typed by primer extension and MALDI-TOF mass spectrometry (MaldiARRAY, Sequenom, San Diego, California) as previously described (Bansal et al, 2002). SNP identity and other linked information may be found in the public databases by using the unique “rs” accession number (NCBI dbSNP, 2003). This SNP had a dropout rate of <0.5% and few Mendelian errors. Where the latter were encountered, the entire family was dropped from analysis. To estimate error rates due to genotyping technical causes, the SNP was typed twice on 3268 DNAs independently at different times. Of 6536 genotypes, there were only seven unresolved errors (not attributable to Mendelian, DNA, or other non-technical causes), which is an error frequency of 0.11%. Of 159 pairs of monozygotic (MZ) twins in the sample set, none were discordant at this SNP, attributable to Mendelian, DNA, or other non-technical causes), which is an error frequency of 0.11%. Of 159 pairs of monozygotic (MZ) twins in the sample set, none were discordant at this SNP, further confirming the low genotyping error rate (one member of each MZ twin pair was set to missing for subsequent association analysis).

Statistical analysis Allelic association between melanoma and the SNP was tested using MENDEL 5.0 (Lange et al, 2001), which implements a measured genotype approach allowing use of family data as well as unrelated individuals. Combined analysis of the published studies was performed via log-linear and ordinal logistic regression analysis using the R statistical package (R Development Core Team, 2003).

This work was supported by grants from the Australian NHMRC (961061, 981339, 199600), the Queensland Cancer Fund, the CRC for Discovery of Genes for Common Diseases, and the US National Cancer Institute (CA88363). We thank Ann Eldridge, Marlene Grace, Megan Campbell and Anjali Egan for technical assistance and the melanoma patients, twins and their families for their cooperation.

DOI: 10.1111/j.0022-202X.2004.23305.x

Manuscript received February 3, 2004; revised March 19, 2004; accepted for publication April 6, 2004

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References


