LETTERS TO THE EDITOR

Skin Type, Melanoma, and Melanocortin 1 Receptor Variants

To the Editor:

We were interested to read the recent paper by Ichii-Jones et al. (1998) published in the Journal of Investigative Dermatology. The authors searched for particular variants of the melanocortin 1 receptor (MC1R) using restriction fragment length polymorphism analysis and performed a case control study with skin type and melanoma. Essentially the authors failed to find any significant relationship between the particular variants they examined and phenotype. We would contend that there are strong associations between particular variants of the MC1R and skin type and possibly with melanoma. We suggest two reasons that may have contributed to the apparent discrepancy between the authors’ results and those previously published by ourselves and others (Valverde et al., 1995, 1996, Box et al., 1997; Smith et al., 1998).

There are over 20 known variants of the MC1R. In our original Nature Genetics paper (Valverde et al., 1995) we detected some but not all of these. As was appropriate for an initial survey, putative associations required confirmation and Ichii-Jones et al.’s paper is a welcome contribution to this. Subsequent work by ourselves and by others has shown that three particular variants, the Arg151Cys, Arg160Trp, and Asp294His variants, are strongly associated with red hair with a majority of individuals with red hair being either compound heterozygotes or homozygotes for one of these three alleles. To this extent red hair approximates to a Mendelian recessive model (Smith et al., 1998). Importantly the relative risks for red hair and possession of one of these alleles are actually quite high (8–15-fold). We would therefore suggest that the results of Ichii-Jones et al. can be accounted for in two ways. First, previous study by ourselves failed to find any association between the codon 92 variant or the codon 84 variant and skin type or hair color (Valverde et al., 1996). This was confirmed in the study by Box et al. (1997) and more recently by Smith et al. (1998). The failure of Ichii-Jones et al. to detect a statistically significant association between the Asp294His variant and hair color or skin type is perhaps more surprising. One possibility is that Ichii-Jones et al. carried out much of their phenotypic assessment in busy outpatient where (as they state) insufficient time meant that not all clinical data were obtained from all patients. Furthermore the authors report that many different individuals carried out the skin typing and assessment of hair color. We think use of standardized hair charts together with a more rigorous assessment of skin type is preferable. We suspect that this is the likely explanation for their failure to detect a relation between the 294 variant and hair color or skin type. The recent publication of evidence of functional impairment of the Arg151Cys variant (Frändberg et al., 1998) together with our own unpublished observations on the Asp294His and Arg151Cys, strongly support a role for these particular variants in the red hair/skin type I phenotype.

In their study, Ichii-Jones et al. (1998) reported that allele frequencies were not different in melanoma cases and controls for any of the three MC1R variants investigated, but that after correction for imbalances in age, gender, and skin type, the association of the Asp84Glu allele with increased risk of melanoma approached significance (p = 0.069, odds ratio = 3.0, 95% CI = 0.9–9.6); however, because of the large number of MC1R variants that exist and because in our previous study on MC1R variants in subjects with melanoma only the second and seventh transmembrane domains were sequenced (Valverde et al., 1996), the question still remains open whether many of the numerous MC1R variants predispose to melanoma, or whether certain variants confer higher risk than others. Subsequently we have examined the entire coding region of the MC1R gene by bidirectional automated sequencing in 26 individuals, 20 of whom were included in our original study and who did not contain the Asp84Glu variant. In addition, the frequency of the Asp84Glu variant was investigated by restriction fragment length polymorphism analysis in another 26 individuals from a similar U.K. population, none of whom were included in our original study. DNA was extracted from blood, paraffin-embedded skin or paraffin-embedded melanoma from subjects with cutaneous melanoma [in our original study variants that were present in the melanoma were also always present in the germline, and chromosome arm 16q where the MC1R gene is located does not seem to be a target for loss of heterozygosity in cutaneous melanoma (Healy et al., 1996)]. MC1R variants were identified in 16 of the 26 melanoma cases that were sequenced for the entire coding region of the MC1R gene [with the Val60Leu variant observed in 11 cases (Table I); however, the Asp84Glu variant was identified in only two of the 32 patients who were not included in our previous study, suggesting that the Asp84Glu variant is not as frequent as previously suspected in melanoma patients, and that this variant is unlikely to be a major risk factor for the development of cutaneous melanoma. The overall frequency of MC1R variants in the 26 melanoma patients in whom the gene was sequenced does not differ significantly from that detected by our group, and recently reported in the Journal of Investigative Dermatology (Smith et al., 1998), in a fair skinned Caucasian population (16 of 26 melanoma cases versus 53 of 71 Irish individuals, Chi² = 0.278, p = 0.597). It seems likely that the variation between the studies is primarily due to population sampling because of the large number of variants that exist in the MC1R gene, and we are reluctant at this stage to attribute any special importance to the association of the Val60Leu variant with melanoma for instance.

We would suggest that, at the current time, sequencing of the entire MC1R gene is essential in investigations relating to many variants with particular phenotypes. Study of selected restriction fragment length polymorphisms may miss significant associations, as we would contend with the Ichii-Jones study. MC1R variants are associated with red hair and fair skin, phenotypic characteristics

Table I. MC1R variants detected by sequencing the entire MC1R coding region in 26 individuals with cutaneous melanoma

<table>
<thead>
<tr>
<th>Variant</th>
<th>Number of cases with variant</th>
</tr>
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<tbody>
<tr>
<td>Val60Leu</td>
<td>11</td>
</tr>
<tr>
<td>Val92Met</td>
<td>3</td>
</tr>
<tr>
<td>Arg142His</td>
<td>2</td>
</tr>
<tr>
<td>Arg151Cys</td>
<td>3</td>
</tr>
<tr>
<td>Arg160Trp</td>
<td>1</td>
</tr>
<tr>
<td>Arg163Gln</td>
<td>1</td>
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that render individuals susceptible to cutaneous melanoma, but further work is required to dissect out whether MC1R variants in melanoma act independently of the skin type phenotype.

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REFERENCES


Reply

Healy et al raise some interesting points in their letter regarding the importance of polymorphism in the melanocortin 1 receptor (MC1R) and its association with skin type, hair color, and susceptibility to melanoma. We have been studying putative associations between polymorphic candidate genes and susceptibility to skin cancer. We have identified allelic variants at several loci that are associated with multiple basal cell carcinomas or a shorter time from first to second presentation (Lear et al., 1996). In some cases, we identified interactions between genotypes and skin type 1 that were associated with a worst clinical outcome. Accordingly, the Valverde et al. (1995) study showing a particularly strong association between allelic variants at the MC1R and red hair and skin type 1 was of considerable interest as a candidate for cutaneous cancers. We initially looked at three of the described alleles, Val92Met, Asp294His, and Asp84Glu, in patients with melanoma. We selected these from the nine alleles described because Val92Met and Asp294His appeared to be relative common and Asp84Glu had been associated with susceptibility to melanoma in a small case control study (Valverde et al., 1996). We studied 306 melanoma cases and 190 controls and attempted to show associations between alleles and skin type and hair color in both groups. In the case group we attempted to also demonstrate differences in allele frequency with tumor type and site (Ichi-Jones et al., 1998).

We failed to confirm the strong association between allelic variants and skin type reported by Valverde et al. (1995). None the less, in the control group we did identify a significant association between skin type and a group comprising all the controls with any one or more of the three alleles studied. Thus, although we did not find the strong association previously reported for individual alleles, our data do support the view that allelic variation at the MC1R is associated with skin type. Importantly, this association was not evident in the melanoma case group.

Healy et al also comment on the association between MC1R allelic variants and hair color. They report that three variants – Arg151Cys, Arg160Trp, and Asp294His – are strongly associated with red hair. The data presented in our study also indicate that Asp294His is associated with red hair in both controls and melanoma cases. This association only approached statistical significance (p = 0.10) in our study (Ichi-Jones et al., 1998). We believe this reflects the relatively small number of red-haired subjects that we were able to recruit. Further studies in a larger patient group have shown a statistically significant association between this allele and red hair.

Overall therefore, we find the association between MC1R alleles and skin type to be less strong than reported by Valverde et al. (1995). We agree that Asp294His (but not Val92Met or Asp84Glu) are associated with red hair. We note the suggestion of Healy et al. that the discrepancies between our data and theirs might result from our inability to properly classify patients as skin type 1 or having red hair. All classifications of skin type were carried out by experienced dermatologists and, anyway, there is general agreement in the literature that classifying patients as skin types 1 or 4 is straightforward (Kricker et al., 1993). Admittedly distinguishing between types 2 and 3 is more problematical. Importantly, in previous studies in patients with skin cancer we have invariably shown expected associations between skin type and clinical outcome (Lear et al., 1996). Similarly, classifying patients as having red hair is straightforward though we accept that the use of standardized charts will help distinguish different shades of red.

The second part of the letter from Healy et al refers to our failure to detect associations between the three alleles studied and susceptibility to melanoma. We are not claiming that the MC1R gene is not involved in the pathogenesis of melanoma. Indeed, in the discussion of our paper we stated that “other allelic variants (possibly some yet unidentified) may be important”. Assessing a candidate gene that demonstrates a relatively large number of alleles is difficult. In our experience large numbers of cases and controls are needed to firstly, assess a susceptibility candidate and secondly, determine whether the gene has any influence on outcome. The influence of most candidate genes so far described is modest (odds ratios about 2–3). Accordingly, our aim has been to identify within case groups, subgroups of patients who are described by particular genotypes and in whom the association between genotypes and susceptibility or outcome is much stronger (Lear et al., 1996). Even with a relatively large study group (190 controls and 306 melanoma cases), it was difficult to assess associations between MC1R alleles and host characteristics. Clearly, larger study groups are needed. These will make sequencing of the entire gene expensive and, anyway, many allelic variants will be very rare and/or will have no obvious functional consequence. Assessing the functional implications of alleles is not always straightforward if the function of the gene is unclear. Thus, whilst we do not disagree that sequencing is an important option, screening of selected alleles in large study groups remains a useful approach. Experiments to assess function can then be focused on alleles shown to be important in molecular epidemiologic studies.

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REFERENCES


Lear JT, Heagerty AHM, Smith A, et al.: Multiple cutaneous basal cell carcinomas: Glutathione S-transferase (GSTM1, GSTT1) and cytochrome P450 (CYP2D6, CYP1A1) polymorphisms influence tumour numbers and accrual. Carcinogenesis 17:1891–1896, 1996


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