Characterization of Melanocortin-1 Receptor Gene Variants in Uveal Melanoma Patients

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PURPOSE. Allelic variations of the melanocortin-1 receptor (MC1R) gene have been linked to red hair and sun-sensitive skin types and may play a role in the susceptibility to develop cutaneous malignant melanoma (CMM). To define the role of MC1R gene in uveal melanoma, a case control study was performed, in which the presence of MC1R gene variations in uveal melanoma patients was compared with that of healthy controls.

METHODS. MC1R gene variants were analyzed in 162 uveal melanoma patients and 255 healthy controls. After genomic DNA was isolated from venous blood, the MC1R gene was amplified by polymerase chain reaction (PCR) and examined for the presence of variants by single-strand conformation polymorphism (SSCP) analysis. Participants were asked to complete a questionnaire regarding skin type, eye color, and hair color.

RESULTS. No disparity was found between the distribution of the MC1R gene variants in both groups. Furthermore, no associations between MC1R genotype and pigment phenotype were found. In contrast to CMM, uveal melanoma patients did not show specific MC1R gene variants. Compared with controls, most uveal melanoma patients had blue eyes (65%, P = 0.060) and skin type III (56%); however, in the uveal melanoma group the presence of dark blond hair was significantly elevated (46%, P = 0.030). These findings are in contrast with studies on CMM, where most patients have skin type II and red/fair hair.

CONCLUSIONS. These data suggest that MC1R variants do not play a role in the susceptibility to develop uveal melanoma. Furthermore, most uveal melanoma patients share phenotypic characteristics that differ from findings in CMM patients. (Invest Ophthalmol Vis Sci. 2001;42:1951–1954)

Uveal melanoma is the most common primary intraocular malignancy in adults, with an annual estimated frequency of six cases per one million subjects in white populations of the Western world.¹ Until now, the genetics of intraocular melanoma have not been extensively studied.² This is in contrast with cutaneous malignant melanoma (CMM), which occurs more frequently and in which several genetic factors have been studied.

Melanomas of the skin and eye arise from melanocytes that have undergone malignant transformation.¹ Uveal melanocytes as well as cutaneous melanocytes originate in the neural crest. During embryonal development they migrate to their respective sites.³ They share several (immuno-)histologic characteristics; however, with respect to cytogenetic aspects and familial occurrences,⁴ they are very different.

Multiple phenotypic risk factors have been identified in the etiology and pathogenesis of CMM, including the number of melanocytic nevi, presence of atypical nevi, pale skin, light blond or red hair, blue eyes, tendency to freckle, and a tendency to burn rather than to tan.⁵⁻¹⁰ The presence of a positive family history is a potential risk factor to develop cutaneous melanoma. In uveal melanoma the importance of phenotypic risk factors is less well defined, and familial involvement is very rare.¹¹ Some studies suggest that the presence of atypical nevi,¹²⁻¹⁴ a light eye color,¹⁵,¹⁶ and the influence of sunlight²,¹⁷,¹⁸ may contribute to the etiology of uveal melanoma. This may suggest that similar genetic factors may be involved in the onset of melanoma of the skin and of the uveal tract, particularly those that influence pigmentation.

Variation in human pigmentation is due to variable amounts of eumelanin (brown/black melanosins) and pheomelanin (red/yellow melanosins) produced by the melanocytes.¹⁹ A large amount of photoprotective eumelanin is found among individuals with a dark skin color (skin type III and IV) and pheomelanin in individuals with a fair skin (skin type I and II). Pheomelanin generates free radicals in response to ultraviolet (UV) radiation, which may contribute to UV-induced skin damage.²⁰,²¹ The melanocortin-1 receptor (MC1R) gene is a regulator of eumelanin production and is located on chromosome 16q24.3. The gene encodes a G-protein–coupled receptor, which is present on the cell membrane of melanocytes and keratinocytes. Binding of α-melanocyte–stimulating hormone (α-MSH) to MC1R stimulates the synthesis of melanosomes and melanin.²²,²³ Point mutations in the MC1R gene will lead to loss of function by changes in ligand binding and inability to stimulate cAMP production. Thereby they affect the pattern of melanogenesis,¹⁹ leading to changes in the ratio of eumelanin/ pheomelanin and possibly stimulating malignant transformation.² The presence of point mutations in one or both alleles of the MC1R gene is a common feature in light skin types (skin type I and II).²⁴⁻²⁶ People with skin types IV and V usually have a wild-type MC1R genotype and rarely carry variants. Three MC1R alleles—Arg151Cys, Arg160Trp, and Asp294His—are statistically associated with red hair,²⁷ and they are also over-represented in individuals with a fair skin type.²⁶ Furthermore, allele Val60Leu is frequently found in dark blond hair.²⁷ In addition to the association of allelic variation of the MC1R gene and pigmentation, several studies suggest that specific human MC1R alleles may be associated with increased CMM susceptibility.²⁸⁻⁵⁰ For example, the presence of Asp84Glu is...
associated with the development of CMM,28 and the presence of one of the above mentioned variants Arg151Cys, Arg160Trp, and Asp294His may double the CMM risk.27

In the present study, we analyzed the role of MC1R gene variants in relation to uveal melanoma. Furthermore, we compared skin type (using the Fitzpatrick classification31), hair color, and eye color between a population of uveal melanoma patients and healthy controls.

**METHODS**

**Patients and Controls**

This study conformed to the tenets of the Declaration of Helsinki, and informed consent was obtained from the participants. The melanoma group consisted of 162 Dutch uveal melanoma patients who visited the Department of Ophthalmology at the Leiden University Medical Center (LUMC) between April 1998 and March 1999. All patients were diagnosed having primary choroidal and/or ciliary body melanoma at the age of 13 to 88 years. In this study, the patients’ ages varied from 26 to 88 years. The control group consisted of 255 unrelated patients who visited the Ophthalmologic Outpatient Clinic of the LUMC between November 1997 and January 1998 (age between 30 and 80 years). Exclusion criteria for both patients and controls were the xeroderma pigmentosum (XP) syndrome and being non-white. Patients and controls who were treated for CMM were also excluded from this study, to prevent a selection bias, as CMM might have the same etiology as uveal melanoma. All participants in this study were asked to complete a questionnaire regarding phenotypic characteristics (skin type by the Fitzpatrick classification,31) eye color, natural hair color at the age of 20, skin color before sun tan, reaction to sun exposure, history of sunburn, and ability to freckle. A score was given for different levels of each parameter, ultimately determining skin type. Skin type I implies fair skin, which always burns, never tans, and only becomes freckled after repeated and prolonged exposure to sunlight. Individuals with skin type II will usually burn, and they tan less than average (mild tanning); people with skin type III will sometimes burn mildly and always tan (moderate tanning); individuals with skin type IV will never burn and will deeply tan after repeated and prolonged exposure to sunlight (intense tanning).

**Detection of MC1R Gene Variants**

Genomic DNA from 162 choroidal and ciliary body melanoma patients and 255 healthy controls was isolated from peripheral blood leukocytes by routine methods.52 The MC1R gene was amplified by polymerase chain reaction (PCR) and analyzed for known variants in the human MC1R gene (Val60Leu, Asp84Glu, Val92Met, Arg142His, Arg151Cys, Ile155Thr, Arg160Trp, Arg163Gln, Pro230Leu, His260Pro, and Asp294His). A specific PCR product of MC1R coding sequence (GenBank accession number X65634) was amplified by PCR in the following reaction: 60 mM Tris.HCl, pH 10.0; 2.0 mM MgCl2; 15 mM (NH4)2SO4; 100 µM each dGTP, dTTP, dATP, and dCTP; 1 µl [α-32P]dCTP (3000 Ci mmol−1); 500 ng of each PCR primer; 2 U Ampli Taq (Perkin Elmer Cetus, Norwalk, CT), and 10% DMSO in a total volume of 100 µl. Ten microliters of reaction mixture was added to 20 ng genomic template DNA. Samples were covered with mineral oil, denatured for 4 minutes at 92°C, and passed through 33 cycles of amplification, consisting of 50 seconds denaturation at 92°C, 50 seconds primer annealing at 60°C, and 2 minutes elongation at 72°C. The amplifications were carried out in 0.5 ml tubes (Perkin Elmer). The DNA sequences of the primers were: F-5’CAACGACTCCCTGCT-GCTG3’ and R-5’TGGCCACAGCCTTAAGGC3’, resulting in a 1018-bp PCR fragment. This resulting fragment was digested by either 2 U Rsal or MspI and was screened for mutations by single-strand conformation polymorphism (SSCP) analysis on a 6% polyacrylamide gel with 10% glycerol. The gels were run at room temperature for 6 hours at 26 W or 16 hours at 20 W for MspI and Rsal digests, respectively.

**RESULTS**

Previous studies showed an association between the occurrence of certain MC1R gene variants and the development of CMM. We wondered whether we could find the same relationship for uveal melanoma.

Analyzing the distribution of MC1R gene variants, we found that 108 (67%) of 162 uveal melanoma patients were carriers of one or two variant alleles in the genotype. Similarly, 177 (69%) of 255 healthy controls were carriers of one or two variant alleles. In 36 (22%) of the 162 uveal melanoma patients, both alleles showed variants. The presence of two variants was found in 51 (20%) of 255 control cases (see Table 1). In both groups, five MC1R gene variants appeared frequently—Val60Leu, Val92Met, Arg151Cys, Arg160Trp, Arg163Gln, but there was no significant difference in the frequency of the different MC1R gene variants between the uveal melanoma patients and the control group (see Table 1).

**Distribution of Skin Type in Uveal Melanoma Patients and Healthy Controls**

Most of the uveal melanoma patients had skin type III (56%), followed by 32% of the patients having skin type II. In the control group, skin type III was present in 49% and skin type II in 40%. No significant differences were found with respect to skin type (P = 0.288).

Table 2 shows the number of variants present in the uveal melanoma patients and the controls, grouped by skin type. MC1R gene variants occurred more frequently in individuals with fairer skin type (I and II) than in those with darker skin type (III and IV). We found that 11 (85%) of 13 uveal melanoma

**Table 1. MC1R Gene Variants in Uveal Melanoma Patients and in Healthy Controls**

<table>
<thead>
<tr>
<th>MC1R Gene Variants</th>
<th>Uveal Melanoma Patients</th>
<th>Healthy Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of variants*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>54 (33.3%)</td>
<td>78 (30.6%)</td>
</tr>
<tr>
<td>1</td>
<td>72 (44.4%)</td>
<td>126 (49.4%)</td>
</tr>
<tr>
<td>2</td>
<td>36 (22.2%)</td>
<td>51 (20.0%)</td>
</tr>
<tr>
<td>Frequency of variants†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val60Leu</td>
<td>32 (9.9%)</td>
<td>48 (9.4%)</td>
</tr>
<tr>
<td>Asp84Glu</td>
<td>5 (0.9%)</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>Val92Met</td>
<td>28 (8.6%)</td>
<td>42 (8.2%)</td>
</tr>
<tr>
<td>Arg142His</td>
<td>2 (0.6%)</td>
<td>3 (0.6%)</td>
</tr>
<tr>
<td>Arg151Cys</td>
<td>19 (5.9%)</td>
<td>28 (5.5%)</td>
</tr>
<tr>
<td>Ile155Thr</td>
<td>1 (0.3%)</td>
<td>3 (0.6%)</td>
</tr>
<tr>
<td>Arg160Trp</td>
<td>37 (11.4%)</td>
<td>55 (10.8%)</td>
</tr>
<tr>
<td>Arg163Gln</td>
<td>14 (4.3%)</td>
<td>23 (4.5%)</td>
</tr>
<tr>
<td>Pro230Leu</td>
<td>1 (0.3%)</td>
<td>5 (0.6%)</td>
</tr>
<tr>
<td>His260Pro</td>
<td>1 (0.3%)</td>
<td>3 (0.6%)</td>
</tr>
<tr>
<td>Asp294His</td>
<td>4 (1.2%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>Other (less frequent variants)</td>
<td>2 (0.6%)</td>
<td>17 (3.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>324 (100%)</td>
<td>510 (100%)</td>
</tr>
</tbody>
</table>

*† Differences were not significant.

**Statistical Analysis**

Allele frequencies, skin type, hair color, and eye color were compared between patients with uveal melanoma and healthy controls using the χ² test. All statistical tests were considered significant at P < 0.05.
patients with skin type I had MC1R gene variants, 36 (69%) of 52 with skin type II, 57 (63%) of 90 with skin type III, and 4 (57%) of 7 with skin type IV. In the controls, we found a similar distribution of the frequency of MC1R gene variants (see Table 2). No significant associations were found between the presence of particular variants of the MC1R gene and eye color (data not shown).

### Distribution of Hair Color in Uveal Melanoma Patients and Healthy Controls

The distribution of hair color in uveal melanoma patients showed no differences compared with the control group ($P = 0.188$; see Table 2). The presence of red hair occurred with the same frequency in both groups: 6% in the uveal melanoma patients and 5% in the controls. The presence of dark blond hair in uveal melanoma patients (46%) is significantly elevated compared with the presence in the control group (34%; $P = 0.050$). There were no significant associations between the presence of MC1R gene variations (or any particular variant) and hair color (data not shown).

### Distribution of Eye Color in Uveal Melanoma Patients and Healthy Controls

The distribution of various eye colors was practically the same in the two groups. Blue-eyed individuals were over-represented in both groups. In our uveal melanoma population, 65% had a blue eye color, compared with 55% in the control group, which approaches significance ($P = 0.060$; see Table 2). We found no significant associations between the number or particular variants of the MC1R gene and eye color (data not shown).

### Discussion

In uveal melanoma the importance of phenotypic risk factors is not well defined. Some studies suggest that susceptibility to develop ocular melanoma may be determined by phenotypic characteristics such as atypical nevi, blue eye color, and the influence of UV light. Interaction between the pigmentary system and UV light may increase the risk of developing uveal melanoma.

In the present study we analyzed whether variants of the human MC1R gene, which are involved in the pathway of melanin formation, may be a risk factor involved in the development of uveal melanoma. Comparing the distribution of the known human MC1R gene variants in white uveal melanoma patients and controls, we found no significant differences. Five variants, Val60Leu, Val92Met, Arg151Cys, Arg160Trp, and Arg163Gln, were found in a high frequency in both groups. These findings are in accordance with other studies on MC1R variant distribution.

In contrast with Smith and Palmer, who reported significant associations of Val60Leu with dark blond hair and with the presence of red hair and a light skin type in relation to Arg151Cys, Arg160Trp, and Asp294His, we did not find such strong associations. In the present study one may wonder whether the lack of significance of associations between MC1R gene variants and these phenotypic characteristics was due to lack of statistical power. With the present sample size, however, there is approximately 80% power to detect a difference of 10% in allele frequency, inclining to rule out a type II error.

The distribution of specific cutaneous melanoma-related MC1R gene variants, for example, Asp84Glu (possibly associated with the development of CMM), Arg151Cys, and Asp294His (probable doubling the CMM risk) is under-represented in our population of uveal melanoma patients compared with the frequencies found in CMM populations.

Most uveal melanoma patients had skin type III (56%), dark blond hair (46%), and blue eyes (65%). Comparing the distribution of skin type in both groups, we did not find significant differences ($<6\%$ difference in skin types I/II). The presence of dark blond hair in our uveal melanoma population was significantly elevated compared with the control group ($P = 0.030$), and the presence of blue eyes approaches significance ($P = 0.060$). The latter is in accordance with the findings of Regan et al., who reported that uveal melanoma are more prevalent among patients with light irises than with dark irises. This may be due to an increased sensitivity to sunlight exposure.

In several studies many phenotypic risk factors have been identified for cutaneous melanoma, including pale skin, red or fair-hair, blue eyes, multiple melanocytic nevi, the presence of atypical nevi, a tendency to freckle, and a tendency to burn rather than to tan. Because of these factors, most of the CMM patients will be red or fair-haired and will have skin type I or II. In summary, comparing our phenotypic results of uveal...
melanoma patients with the above-mentioned phenotypic characteristics of cutaneous melanoma patients, we can conclude that the uveal melanoma population differs significantly from the CMM population. The results of this study show that MC1R gene variants do not play a role in the susceptibility to develop uveal melanoma.

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References