Melanocortin 1 Receptor (*MC1R*) Gene Variants are Associated with an Increased Risk for Cutaneous Melanoma Which is Largely Independent of Skin Type and Hair Color

Cornelis Kennedy,* Jeanet ter Huurne,* Marjo Berkhout,* Nelleke Gruis,*† Maarten Bastiaens,* W. Bergman,* R. Willemze,* and Jan Nico Bouwes Bavinck*

Departments of *Dermatology and †Human and Clinical Genetics, Leiden University Medical Center, RC Leiden, the Netherlands

Individuals carrying melanocortin 1 receptor gene variants have an increased risk for the development of cutaneous melanoma. Melanocortin 1 receptor gene variants are also associated with other risk factors for melanoma such as fair skin and red hair. We evaluated the relationship of melanocortin 1 receptor gene variants, fair skin, red hair and the development of melanoma in 123 patients with cutaneous melanoma and 385 control subjects. To analyze the association between melanocortin 1 receptor gene variants and skin type or hair color we also made use of 453 patients with nonmelanoma skin cancer. We analyzed the coding sequence of the melanocortin 1 receptor gene region by single-stranded conformation polymorphism analysis, followed by DNA sequence analysis. Risk of melanoma dependent on the various melanocortin 1 receptor variant alleles was estimated by exposure odds ratios. The analyses of all different melanocortin 1 receptor gene variants combined, showed that the presence of melanocortin 1 receptor gene variants amounted to a higher melanoma risk, which, in stratified analyses, was independent of skin type and hair color. The odds ratios after adjusting for skin type were 3.6 (95% CI 1.7–7.2) for two variants and 2.7 (95% CI 1.5–5.1) for one variant, respectively. Compound heterozygotes and homozygotes for the Val60Leu, Val92Met, Arg142His, Arg151Cys, Arg160Trp, Arg163Gln, and His260Pro variants had odds ratios of about 4 to develop melanoma, whereas heterozygotes for these variants had half the risk. The presence of the melanocortin 1 receptor gene variant Asp84Glu appeared to impose the highest risk for cutaneous melanoma with odds ratios of 16.1 (95% CI 2.3–139.0) and 8.1 (95% CI 1.2–55.9) in compound heterozygotes and heterozygotes, respectively. The broad confidence intervals, when the different variants were analyzed separately, however, do not allow drawing definite conclusions about the magnitude of these risks. Of the more frequently occurring melanocortin 1 receptor variant alleles the Asp84Glu, Arg142His, Arg151Cys, Arg160Trp, His260Pro, and Asp294His variants were strongly associated with both fair skin and red hair. The Val60Leu, Val92Met, and Arg163Gln variant alleles, however, were only weakly or not associated with fair skin type and/or red hair, which further illustrates the finding that skin type, hair color, and melanoma are independent outcomes of the presence of melanocortin 1 receptor gene variants. We conclude that numerous melanocortin 1 receptor variants predispose to cutaneous melanoma and that possibly the Asp84Glu variant confers the highest risk. This predisposition is largely independent of skin type and hair color. ***Key words: epidemiology/melanoma/melanocortin 1 receptor gene. J Invest Dermatol 117:294–300, 2001***

Variants of the melanocortin 1 receptor-gene (*MC1R* gene) are associated with an increased risk for the development of cutaneous melanoma (Valverde *et al*, 1996; Rees and Healy, 1997; Healy *et al*, 1999; Palmer *et al*, 2000). *MC1R* gene variants are also associated with phenotypical features such as fair skin and red hair that carry a higher risk for both melanoma and nonmelanoma skin cancer (Bliss *et al*, 1995; Valverde *et al*, 1995; Katsambas and Nicolaidou, 1996; Rees and Healy, 1997; Flanagan *et al*, 2000; Harding *et al*, 2000; Healy *et al*, 2000; Palmer *et al*, 2000; Bastiaens *et al*, 2001; Box *et al*, 2001). Furthermore, the *MC1R* gene is closely associated with sun sensitivity in the majority of individuals without red hair (Healy *et al*, 2000). Considering these associations the *MC1R* gene, therefore, is of substantial importance as a susceptibility gene for sunburn, photoaging, and cutaneous melanoma (Healy *et al*, 2000).

The MC1 receptor located on chromosome 16q24.3 is a seven-pass transmembrane, G-protein coupled receptor that has high affinity for α-melanocyte-stimulating hormone (α-MSH) (Chhajlani and Wikberg, 1992; Mountjoy *et al*, 1992; Healy *et al*, 1998). Different variants of the *MC1R* gene can lead to a change in

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Reprint requests to: Dr. Jan Nico Bouwes Bavinck, Department of Dermatology, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands. Email: dermatol@euronet.nl

Abbreviations: MC1 receptor, melanocortin-1 receptor; α-MSH, α-melanocyte stimulating hormone.

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ligand binding, a change in function, or loss of function of the MC1 receptor (Cone *et al*, 1996; Valverde *et al*, 1996; Box *et al*, 1997; Koppula *et al*, 1997).

The effect of the MC1 receptor on melanogenesis is mediated through the activation of adenylyl cyclase to elevate cyclic adenosine monophosphate levels after binding with α-MSH and adrenocorticotropic hormone (Mountjoy *et al*, 1992). Generally, stimulation with α-MSH leads to eumelanin synthesis which is considered predominantly photoprotective, but variations in the *MC1R* gene of which more than 20 have been reported may lead to a different response (Rees and Healy, 1997; Healy *et al*, 1998). Loss of function variants may lead to reduced cyclic adenosine monophosphate production after stimulation with α-MSH resulting in increased levels of pheomelanin which is predominantly found in individuals with fair skin and red hair and which may contribute to an increased cancer risk by generating free radicals following ultraviolet exposure (Burchill *et al*, 1986; Chhajlani and Wikberg, 1992; Mountjoy *et al*, 1992; Barsh, 1996; Suzuki *et al*, 1996; Frändberg *et al*, 1998; Smith *et al*, 1998; Schiöth *et al*, 1999).

Several studies have specifically tried to elucidate which *MC1R* gene variants are of particular interest in the development of cutaneous melanoma (Valverde *et al*, 1996; Ichii-Jones *et al*, 1998; Palmer *et al*, 2000). A limitation of these studies, however, is that only a limited number of particular *MC1R* gene variants was studied in sometimes small numbers of patients and controls.

Valverde *et al* (1996) studied known MC1R variants in the second (codons 64–106: e.g., Asp84Glu, Asn91Asp, Val92Met) and seventh (codons 278–300: e.g., Asp294His) transmembrane domains in 43 melanoma cases and 44 controls, and they reported that MC1R variants were more common in melanoma cases than controls. They found that the Asp84Glu accounted for most of this difference as this variant was present in 10 of the melanoma cases and not in the controls. In Valverde *et al*'s (1996) study, the contribution of MC1 receptor alleles seemed largely independent of skin type.

A study by Ichii-Jones *et al* (1998) evaluated the Asp84Glu, Val92Met, and Asp294His variants in a large but heterogeneous population consisting of 306 cases with *in situ* melanoma, lentigo melanoma, nodular melanoma, and superficial spreading melanoma in comparison with 190 controls. They were not able to detect a difference between the melanoma cases and controls. However, after correction for imbalances with age, gender, and skin type, the association of the Asp84Glu variant with increased risk of melanoma approached significance ($p = 0.069$, odds ratio = 3.0, 95% CI 0.9–9.6).

Palmer *et al* (2000) studied six MC1R variants (Val60Leu, Asp84Glu, Arg151Cys, Arg160Trp, Asp294His, and 537insC) in 460 cases selected from high-risk, intermediate-risk and low-risk families with familial melanoma in comparison with 399 controls. The *MC1R* gene variants Arg151Cys, Arg160Trp, and Asp294His were more common in individuals with cutaneous malignant melanoma. The odds of melanoma increased 2.2-fold (95% CI 1.6–3.0) in individuals carrying one of these three variants, and it increased 4.1-fold (95% CI 2.1–7.9) if at least two variants were carried. Among pale-skinned individuals alone, this association between MC1R variants and cutaneous melanoma was absent, but it persisted among those reporting a medium or olive/dark complexion. In Palmer *et al*'s (2000) study no association could be demonstrated between the Asp84Glu variant and cutaneous melanoma.

The discrepancies in the different studies prompted us to investigate the relationship between *MC1R* gene variants and cutaneous melanoma in a cohort of Dutch patients. The question remains open whether many of the numerous MC1R variants predispose to cutaneous melanoma, or whether certain variants confer higher risk than others (Healy *et al*, 1999). Specifically the role of the Asp84Glu variant merits further study as this variant is not as frequent as previously suspected in melanoma patients (Valverde *et al*, 1996; Healy *et al*, 1999). The specific aims of this study therefore were to assess whether: (i) the association between *MC1R* gene variants and cutaneous melanoma also exists in a large cohort of Dutch patients, and (ii) with which variants this association is most prominent. We analyzed the coding region of the *MC1R* gene region by single-stranded conformation polymorphism analysis, followed by DNA sequence analysis.

MATERIALS AND METHODS

Study population The Leiden Skin Cancer study was initiated in 1997 with the objective to assess the relative risks associated with known and suspected risk factors for skin cancer in a Dutch cohort of patients with melanoma and nonmelanoma skin cancer compared with control persons (Bastiaens *et al*, 2001; De Hertog *et al*, 2001). Except for the analyses to calculate the association between MC1R variants and skin type or hair color, the study reported here was restricted to nonfamilial cutaneous melanoma and controls. The Medical Ethics Committee approved the study protocol and informed consent was obtained from all participants in the study.

Control subjects, aged 30–80 y of age were recruited for this study at the ophthalmology outpatient department at the Leiden University Medical Center. Ophthalmology patients were chosen to represent the controls because this group consisted of patients visiting the same university hospital and were a good representation of patients living in the same demographic area. Furthermore, eye conditions are usually not related to factors that have been identified as being a risk factor for skin cancer in general. Controls were excluded when they had an intraocular melanoma or any type of skin cancer in their medical history. Both cases and controls were excluded from participation in this study when they were transplant recipients or suffered conditions associated with an increased risk for skin cancers such as xeroderma pigmentosum and Gorlin's syndrome (basal cell nevus syndrome) or when they could be classified as being skin type V or VI according to Fitzpatrick's skin type classification, which is associated with a decreased risk for skin cancer (Fitzpatrick, 1988).

Collection of data on risk factors for cutaneous melanoma The visit at the dermatology outpatient clinic consisted of a standardized personal interview by a trained interviewer. Participants were not informed about our research objectives and interviewer or dermatologists were not informed about the case or control status of the participating subjects. Following the interview a total skin examination was performed by a board-certified dermatologist who was not informed about the patient's case or control status.

The physical examination was performed according to a set protocol and data were entered on to a preprinted examination sheet. The dermatologist was requested to collect information on eye and hair color, the amount of freckles and lentigines, number and localization of normal and clinically atypical nevi and the presence of clinically manifest skin cancer. Skin types were assessed as always burn, never tan (skin type I); always burn, then tan (skin type II); always tan, sometimes burn (skin type III); and always tan, never burn (skin type IV) (Fitzpatrick TB, 1988). Hair color was retrospectively assessed as hair color at age 20 and was classified as black, brown, dark blond, light blond or red. Eye color was determined as blue, green, brown, or gray.

Upon completion of the examination, participants were requested to deliver a blood sample for *MC1R* gene analyses.

Detection of *MC1R* gene variants Genomic DNA was isolated from peripheral blood leukocytes by routine methods (Miller *et al*, 1988). A specific polymerase chain reaction (PCR) product of MC1R coding sequence (GenBank accession number X65634) was digested by either 2 U *Rsa*I or *Msp*I and were screened for mutations by single-stranded conformation polymorphism analysis (Orita *et al*, 1989) on a 6% polyacrylamide gel with 10% glycerol. With this technique, the gels were run at room temperature for 6 h at 28 W or 16 h at 20 W for *Msp*I and *Rsa*I digests, respectively.

PCR reaction mixtures 60 mM Tris–HCl, pH 10.0; 2.0 mM $MgCl_2$; 15 mM $(NH_4)_2SO_4$; 100 μM each deoxyguanosine triphosphate, deoxythymidine triphosphate, deoxyadenosine triphosphate, deoxycytidine triphosphate; 1 μl [α-^{32}P]deoxycytidine triphosphate (3000 Ci per mmol), 500 ng of each PCR primer, 2 U AmpliTaq (Perkin Elmer Cetus, PE Biosystems, Nieuwekerk, Netherlands); and 10% dimethyl sulfoxide in a total volume of 100 μl. A 10 μl reaction was added to 50 ng genomic template DNA. Samples were covered with mineral oil, denatured for 4 min at 92°C, and passed through 33 cycles of amplification, consisting of 50 s denaturation at 92°C, 50 s primer annealing at 58°C, 2 min elongation at 72°C. The amplifications were carried out in

Table I. Characteristics of the patients with melanoma and controls

	Controls (n = 385)	Melanoma (n = 123)
Gender N (%)		
Women	222 (57.7)	76 (61.8)
Men	163 (42.3)	47 (38.2)
Age (y)		
Mean ± SD	58.5 ± 11.2	49.3 ± 12.1
Range	28.6–79.9	24.1–77.5
Skin type N (%)		
IV: tan/never burn	24 (6.2)	3 (2.5)
III: tan/sometimes burn	181 (47.0)	34 (27.6)
II: burn/then tan	155 (40.3)	76 (61.8)
I: burn/never tan	25 (6.5)	10 (8.1)
Hair color N (%)		
Black	27 (7.0)	0 (0)
Brown	97 (25.2)	31 (25.2)
Dark blond	143 (37.1)	41 (33.3)
Light blond	96 (24.9)	40 (32.5)
Red	20 (5.2)	9 (7.3)
Not recorded	2 (0.6)	2 (1.7)

0.5 ml tubes (Perkin Elmer). The DNA sequences of the primers were: F-5′CAACGACTCCTTCCTGCTTC3′ and R-5′TGCCCAGCACAC-TTAAAGC3′, resulting in a 1018 bp PCR fragment.

Sequence analysis DNA samples for sequencing were obtained by PCR as described above with M13-tailed MC1R primers. M13MC1R-F-5′TGTAAAACGACGGCCAGTCAACGACTCCTTCCTGCTTC3′ and M13MC1R-IR-5′CAGGAAACAGCTATGACCATGAGTCACG-ATGCTGTGG-TAGC3′ resulted in a 542 bp fragment. The primers M13MC1R-IF-5′GACGTTGTAAACGACGGCCAGTACCTGCAGCT-CCATGCTG-TC3′ and M13MC1R-R-5′CAGGAAACAGCTATGA-CCATGATGCCCAGCACACTTAAAGC3′ resulted in a 661 bp fragment.

Sequence analysis was performed on an ABI-377 automated DNA sequencer using Big-Dye Terminator cycle sequencing kits (Perkin Elmer).

Statistical analysis and strategy Chi-square analysis was used to compare *MC1R* gene variant frequencies in malignant melanoma patients and controls for different skin types and hair colors. Odds ratios (OR) with 95% confidence intervals (CI) were calculated as an estimation of the relative risks of cutaneous melanoma for the different *MC1R* gene variants. Our analyses revealed that almost all frequently occurring variants were associated with an increased risk of melanoma, therefore all different *MC1R* gene variants were combined. Crude OR and Mantel–Haenszel adjusted OR with a 95% confidence were calculated to estimate the relative risk of malignant melanoma in relation to all MC1R genotypes combined. Skin type and hair color were considered to be possible confounding factors in the analyses between the *MC1R* gene variants and melanoma. A Mantel–Haenszel weighted odds ratio was calculated from the results of the different skin type strata or hair color strata to remove potential confounding caused by skin type or hair color.

RESULTS

Composition of study population The final series for analysis comprised 508 subjects: 123 patients with a history of cutaneous melanoma and 385 control subjects. **Table I** shows some basic characteristics of the study population. The majority of both patients with cutaneous melanoma and controls were female. The mean age of the examined melanoma patients was younger than in the control group. Black-haired individuals were only encountered in the control group, whereas light blond and red hair color were overrepresented in the melanoma group. The distribution of the different eye colors (brown, gray, green, and blue) was not different between the melanoma patients and control group (data not shown). Freckles and solar lentigines were more prevalent among melanoma patients and the mean number of both common nevi and atypical nevi was also significantly higher in this group (data not shown).

Not all MC1R variant alleles are associated with fair skin type and red hair For the analyses of the association between MC1R variant alleles and skin type or hair color we extended the group of 123 patients with melanoma and 385 control subjects with 453 patients with nonmelanoma skin cancer. The basic characteristics of the patients with nonmelanoma skin cancer and detailed analyses of this type of skin cancer are described elsewhere (Bastiaens *et al*, 2001; De Hertog *et al*, 2001). Of these 961 persons, 261 (27.2%) had no MC1R variant alleles, 441 (45.9%) one MC1R variant allele, and 259 (26.9%) two MC1R variant alleles. The group with two variant alleles comprised of 35 homozygotes (6 × Val60Leu who did not have red hair, 9 × Val92Met who did not have red hair, 1 × Arg142His who had red hair, 5 × Arg151Cys who all had red hair, 13 × Arg160Trp of whom five had red hair, and 1 × Arg163Gln who did not have red hair) and 224 compound heterozygotes, i.e., two different variant alleles on two separate genes. In total, 27 different MC1R–MC1R variant alleles were found (**Table IIa**).

Table II(**a**, **b**) illustrate the distribution of MC1R variant alleles among the different skin types and hair colors. Of the more frequently occurring MC1R variant alleles the OR for the Asp84Glu, Arg142His, Arg151Cys, Arg160Trp, His260Pro and Asp294His variants to have fair skin and/or red hair were higher than 2.5. The OR for the Val60Leu, Val92Met, and Arg163Gln variant alleles, however, to have fair skin type and/or red hair were lower than 2 (**Table IIa, b**).

Numerous *MC1R* gene variants are associated with melanoma, which is largely independent of skin type and hair color The distribution of MC1R variant alleles among patients with melanoma compared with controls is depicted in **Table II**(***c***). The majority of the most frequently occurring MC1R variant alleles were significantly associated with a history of malignant melanoma. The distribution of MC1R variant alleles in patients with nonmelanoma skin cancer is also provided in **Table II**(***c***). Almost all frequently occurring MC1R variant alleles were also significantly associated with a history of nonmelanoma skin cancer. More detailed information about the association of MC1R variant alleles and nonmelanoma skin cancer has been reported elsewhere (Bastiaens *et al*, 2001).

Compound heterozygotes and homozygotes for the Val60Leu, Val92Met, Arg142His, Arg151Cys, Arg160Trp, Arg163Gln, and His260Pro variants had OR of about 4 to develop melanoma, whereas heterozygotes for these variants had half the risk (**Table III**). Adjustment for skin type did not materially alter these OR (**Table III**). The presence of the *MC1R* gene variant Asp84Glu appeared to impose the highest risk for cutaneous melanoma with OR of 16.1 (95% CI 2.3–139.0) and 8.1 (95% CI 1.2–55.9) in compound heterozygotes and heterozygotes, respectively, but the broad CI do not allow to draw definite conclusions about the magnitude of these risks.

Almost all frequently occurring variant alleles were associated with melanoma. Of the more frequently occurring MC1R variant alleles, however, only the Asp84Glu, Arg142His, Arg151Cys, Arg160Trp, His260Pro, and Asp294His variants were strongly associated with both fair skin and red hair. The Val60Leu, Val92Met, and Arg163Gln variant alleles, were much weaker or not at all associated with fair skin type and/or red hair. The different associations of *MC1R* gene variants with skin type and hair color on one side and melanoma on the other side form a strong argument that these pigmentation characteristics and melanoma are independent outcomes of the presence of *MC1R* gene variants.

To investigate further whether the association between *MC1R* gene variants and melanoma is independent of skin type and hair color, all different *MC1R* gene variants were combined. **Table IV** shows the nonadjusted risk of cutaneous melanoma dependent on

Table II. (*a*) Not all MC1R variant alleles are equally strong associated with fair skin type[a]

Variant[b]	Total no. (n = 1922)	Skin type IV (n = 102) N (%)	Skin type III (n = 764) N (%)	Skin type II (n = 866) N (%)	Skin type I (n = 190) N (%)	OR (95% CI) Skin type I + II *vs* skin type III + IV
Wild type	963 (50.1)	65 (63.7)	459 (60.1)	383 (44.2)	56 (29.5)	1
Val60Leu (178G→T)	179 (9.3)	11 (10.8)	67 (8.8)	84 (9.7)	17 (8.9)	1.5 (1.1–2.1)
Asp84Glu (252C→A)	32 (1.7)	1 (1.0)	7 (0.9)	21 (2.4)	3 (1.6)	3.6 (1.6–8.1)
Val92Met (284G→A)	171 (8.9)	6 (5.9)	64 (8.4)	78 (9.0)	23 (12.1)	1.7 (1.2–2.4)
Arg142His (425G→A)	16 (0.8)	0 (0.0)	4 (0.5)	7 (0.8)	5 (2.6)	3.6 (2.4–5.4)
Arg151Cys (451C→T)	132 (6.9)	1 (1.0)	32 (4.2)	73 (8.4)	26 (13.7)	3.6 (2.4–5.4)
Arg160Trp (478C→T)	224 (11.7)	8 (7.8)	57 (7.5)	121 (14.0)	38 (20.0)	2.9 (2.1–4.0)
Arg163Gln (488G→A)	89 (4.6)	4 (3.9)	33 (4.3)	44 (5.1)	8 (4.2)	1.7 (1.1–2.6)
His260Pro (779 A→C)	19 (1.0)	1 (1.0)	5 (0.6)	10 (1.2)	3 (1.6)	2.6 (0.97–6.9)
Asp294His (880G→C)	20 (1.0)	0 (0.0)	6 (0.8)	9 (1.0)	5 (2.6)	2.8 (1.1–7.3)
Other variants[c]	77 (4.0)	5 (4.9)	30 (3.9)	36 (4.2)	6 (3.2)	1.4 (0.90–2.3)

[a]n = number of alleles.
[b]Between brackets the nucleotide change is shown.
[c]< 0.5% of total alleles: Pro18Ala (52C →G), Ala81Pro (241G →C), Thr95Met (284C →T), Gly104Ser (310G →A), Arg151Arg (453C →G), Ile155Thr (925T →C), Val173del, Val174Ile (981G →A), Pro230Leu (689C →T), Pro230Pro (690G →A), Gln233Gln (699G →A), Ile264Ile (792C →T), Lys278Glu (832A →G), Asn279Ser (835A →G), Asn279Lys (836C →A), 86InsA, 537InsC, *2C →T.

(*b*) Not all MC1R variant alleles are associated with red hair[a]

Variant[b]	Hair color Black (n = 98) N (%)	Hair color Brown (n = 414) N (%)	Hair color Dark blond (n = 692) N (%)	Hair color Light blond (n = 568) N (%)	Hair color Red (n = 142) N (%)	OR (95% CI) Red hair *vs* other hair colors
Wild type	59 (60.2)	213 (51.5)	391 (56.5)	276 (48.6)	19 (13.5)	1
Val60Leu	9 (9.2)	52 (12.6)	61 (8.8)	48 (8.5)	8 (5.6)	2.3 (1.0–5.4)
Asp84Glu	1 (1.0)	10 (2.4)	9 (1.3)	9 (1.6)	3 (2.1)	5.1 (1.4–18.3)
Val92Met	6 (6.1)	41 (9.9)	59 (8.5)	62 (10.9)	3 (2.1)	0.88 (0.26–3.0)
Arg142His	0 (0.0)	1 (0.2)	3 (0.4)	4 (0.7)	8 (5.6)	49.2 (16.8–145.5)
Arg151Cys	4 (4.1)	22 (5.3)	36 (5.2)	31 (5.5)	39 (27.5)	20.7 (11.5–37.3)
Arg160Trp	6 (6.1)	34 (8.2)	69 (10.0)	69 (12.1)	45 (31.7)	12.5 (7.1–21.9)
Arg163Gln	8 (8.2)	22 (5.3)	23 (3.3)	33 (5.8)	3 (2.1)	1.7 (0.50–5.9)
His260Pro	1 (1.0)	3 (0.7)	8 (1.2)	3 (0.5)	3 (2.1)	9.9 (2.6–37.0)
Asp294His	0 (0.0)	2 (0.5)	5 (0.7)	9 (1.6)	4 (2.8)	12.4 (3.8–40.5)
Other variants[c]	4 (4.1)	14 (3.4)	28 (4.1)	24 (4.2)	7 (4.9)	4.9 (2.0–12.2)

See key see footnote for **Table II(*a*)**.

(*c*) MC1R variant alleles are more frequently present among patients with melanoma or non-melanoma skin cancer compared with controls[a]

Variant[b]	Controls (n = 770) N (%)	Non-melanoma skin cancer (n = 906) N (%)	Malignant melanoma (n = 246) N (%)	OR (95% CI) non-melanoma skin cancer *vs* controls	OR (95% CI) malignant melanoma *vs* controls
Wild type	448 (58.2)	419 (46.2)	96 (39.0)	1	1
Val60Leu	64 (8.3)	90 (9.9)	25 (10.2)	1.5 (1.1–2.1)	1.8 (1.1–3.0)
Asp84Glu	7 (0.9)	17 (1.9)	8 (3.3)	2.6 (1.1–6.3)	5.3 (1.9–15.1)
Val92Met	53 (6.9)	93 (10.3)	25 (10.2)	1.9 (1.3–2.7)	2.2 (1.3–3.7)
Arg142His	6 (0.8)	7 (0.8)	3 (1.2)	1.2 (0.42–3.7)	2.3 (0.57–9.5)
Arg151Cys	37 (4.8)	75 (8.3)	20 (8.1)	2.1 (1.4–3.3)	2.5 (1.4–4.5)
Arg160Trp	81 (10.5)	108 (11.9)	35 (14.2)	1.4 (1.0–2.0)	2.0 (1.3–3.2)
Arg163Gln	38 (4.9)	36 (4.0)	15 (6.1)	1.0 (0.63–1.6)	1.8 (0.97–3.5)
His260Pro	3 (0.4)	10 (1.1)	6 (2.4)	3.6 (0.97–13.0)	9.3 (2.3–38.0)
Asp294His	5 (0.7)	13 (1.4)	2 (0.8)	2.8 (0.98–7.9)	1.9 (0.36–9.8)
Other variants[c]	28 (3.6)	38 (4.2)	11 (4.5)	1.5 (0.87–2.4)	1.8 (0.88–3.8)

See key see footnote for **Table II(*a*)**.

MC1R gene variants and the risks stratified according to and adjusted for skin type and hair color. All *MC1R* gene variants combined were also significantly associated with the development of melanoma. Carriers of two variant alleles had a relative risk for melanoma of 4.8 (95% CI 2.5–9.4) compared with carriers of two wild-type alleles, and carriers of one variant allele had again half the

Table III. Risk for cutaneous melanoma for each *MC1R* gene variant

Variant	(A) Heterozygotes (B) Compound heterozygotes and homozygotes	Controls (N = 385) N (%)	Melanoma (N = 123) N (%)	Non-adjusted OR (95% CI)	OR (95% CI) adjusted for skin type
Wild-type homozygotes		137 (35.6)	17 (13.8)	1	1
Val60Leu	A	42 (10.9)	14 (11.4)	2.7 (1.1–6.3)	2.7 (1.1–6.3)
	B	20 (5.2)	10 (8.1)	4.0 (1.5–11.0)	3.2 (1.1–8.9)
Asp84Glu[a]	A	3 (0.8)	3 (2.4)	8.1 (1.2–55.9)	10.4 (1.2–68.5)
	B	2 (0.5)	4 (3.3)	16.1 (2.3–139.0)	Insufficient data
Val92Met	A	31 (8.1)	9 (7.3)	2.3 (0.87–6.2)	2.2 (0.83–6.1)
	B	22 (5.7)	14 (11.4)	5.1 (2.1–12.9)	4.0 (1.4–10.1)
Arg142His[a]	A	3 (0.8)	0	0 (0–19.9)	Insufficient data
	B	3 (0.8)	2 (1.6)	5.4 (0.58–44.0)	4.3 (0.41–35.6)
Arg151Cys[a]	A	19 (4.9)	3 (2.4)	1.3 (0.27–5.3)	1.2 (0.25–5.0)
	B	18 (4.7)	16 (13.0)	7.2 (2.9–18.1)	5.6 (2.2–16.2)
Arg160Trp[a]	A	45 (11.7)	15 (12.2)	2.7 (1.2–6.2)	2.3 (0.97–5.4)
	B	31 (8.1)	17 (13.8)	4.4 (1.9–10.3)	3.4 (1.4–8.5)
Arg163Gln	A	18 (4.7)	6 (4.9)	2.7 (0.82–8.5)	2.6 (0.81–8.5)
	B	18 (4.7)	9 (7.3)	4.0 (1.4–11.4)	3.5 (1.1–9.6)
His260Pro[a]	A	2 (0.5)	5 (4.1)	20.2 (3.1–164.7)	17.3 (2.1–126.8)
	B	1 (0.3)	1 (0.8)	8.1 (0.0–312.5)	10.0 (0.19–325.7)
Asp294His[a]	A	4 (1.0)	2 (1.6)	4.0 (0.47–28.8)	4.1 (0.42–28.7)
	B	1 (0.3)	0	0 (0–148.2)	Insufficient data

[a]Variant alleles which are strongly associated with both fair skin type and red hair color.

Table IV. Risk of melanoma dependent on *MC1R* gene variants stratified according to skin type and red hair color[a]

	All groups together		Skin type III and IV		Skin type I and II		
	Controls N (%)	Melanoma N (%)	Controls N (%) N (%)	Melanoma N (%)	Controls N (%)	Melanoma N (%)	Pooled OR
Wt/wt	137 (35.6)	17 (13.8)	87 (42.4)	8 (21.6)	50 (27.8)	9 (10.5)	
Wt/var	174 (45.2)	62 (50.4)	95 (46.4)	24 (64.9)	79 (43.9)	38 (44.2)	
Var/var	74 (19.2)	44 (35.8)	23 (11.2)	5 (13.5)	51 (28.3)	39 (45.3)	
OR1[b]	2.9 (1.6-5.4)	2.8 (1.1-7.1)	2.7 (1.1-6.5)	2.7 (1.5-5.1)			
OR2[b]	4.8 (2.5-9.4)	2.4 (0.60-9.1)	4.3 (1.8-10.6)	3.6 (1.7-7.2)			

	All groups together		Non-red hair colors		Red hair color		
	Controls N (%)	Melanoma N (%)	Controls N (%) N (%)	Melanoma N (%)	Controls N (%)	Melanoma N (%)	Pooled OR
Wt/wt	136 (35.5)	16 (13.2)	136 (37.4)	16 (14.3)	0	0	
Wt/var	173 (45.2)	62 (51.2)	169 (46.6)	61 (54.5)	4 (20.0)	1 (11.1)	
Var/var	74 (19.3)	43 (35.6)	58 (16.0)	35 (31.2)	16 (80.0)	8 (88.9)	
OR1[b]	3.1 (1.6-5.8)	3.1 (1.6-5.8)	Insufficient data	3.1 (1.6-5.8)			
OR2[b]	4.9 (2.5-9.9)	5.1 (2.5-10.6)	Insufficient data	5.1 (2.5-10.6)			

[a]Wt represents wild type allele; Var represents variant allele.
[b]OR1: odds ratio comparing wt/var (heterozygotes) to wt/wt (wild type homozygotes); OR2: odds ratio comparing var/var (compound heterozygotes and homozygotes for one variant) to wt/wt; pooled OR are adjusted for skin type or hair color using Mantel–Haenszel weighted odds ratio: OR are indicated with 95% CI in all rows.

risk (**Table IV**). After adjustment for skin type the association between *MC1R* gene variants and the occurrence of melanoma remained, suggesting that *MC1R* gene variants are a risk factor for the development of melanoma independent of skin type. Similarly, adjustment for red hair did not materially influence this association (**Table IV**). Unfortunately, the group of persons with red hair was too small to attribute significance to the distribution of MC1R variant alleles within this subgroup. Significant associations between *MC1R* gene variants and melanoma, however, were seen in each of the strata with brown, dark blond, and light blond hair (data not shown).

Conversely, individuals with skin type I and II had a relative risk for melanoma of 2.7 (95% CI 1.7–4.2) compared with skin type III and IV. After adjustment for the presence of *MC1R* gene variants the association between skin type and the risk for melanoma remained (data not shown), indicating that also the association between skin type and melanoma is independent of the presence of *MC1R* gene variants.

DISCUSSION

This study confirmed the association of MC1R gene variants with the occurrence of cutaneous melanoma as was reported by others (Valverde *et al*, 1996; Palmer *et al*, 2000). Additionally, it was shown that numerous frequently occurring *MC1R* gene variants predispose to cutaneous melanoma. Despite small numbers the Asp84Glu allele appeared to be associated with the highest risk of cutaneous melanoma in this cohort of Dutch patients. This finding is in accordance with Valverde's study who reported that the Asp84Glu allele seemed to be of particular significance in melanoma cases (Valverde *et al*, 1996). The broad CI, however, do not allow to us to draw definite conclusions about the magnitude of risk of melanoma imposed by the Asp84Glu allele.

Although the strong association of *MC1R* gene variants with fair skin type and red hair (Valverde *et al*, 1995; Flanagan *et al*, 2000; Harding *et al*, 2000; Healy *et al*, 2000; Palmer *et al*, 2000; Box *et al*, 2001) could be confirmed convincingly, our analyses also showed that skin type and hair color did not materially influence the association between *MC1R* gene variants and the occurrence of cutaneous melanoma. Separate analyses of the more commonly occurring MC1R variant alleles showed that they were all associated with cutaneous melanoma, although, because of the low frequency of some variant alleles, statistical significance was not always reached. Not all MC1R variant alleles, which were associated with melanoma, however, were also associated with fair skin and/or red hair. Specifically, the Val60Leu, Val92Met, and Arg163Gln variant alleles were weaker associated or not associated with fair skin type and/or red hair, which further illustrates the finding that these pigmentation characteristics and malignant melanoma are independent outcomes of the presence of *MC1R* gene variants.

Still another argument that in cutaneous melanoma the influence of the MC1R signaling pathway may be additional to any effects on the pigmentary phenotype comes from recent findings that *MC1R* gene variants are also associated with an increased risk of nonpigmented skin cancers, such as squamous cell and basal cell carcinoma (**Table II*c***; Bastiaens *et al*, 2001; Rees and Healy, 1997; Box *et al*, 2001). All these findings together strongly suggest that *MC1R* gene variants in cutaneous melanoma act largely independently of the skin type or hair color phenotype, but we need further studies to ultimately prove or disprove this hypothesis.

The exact mechanisms underlying the increased risk of individuals carrying *MC1R* gene variants to develop melanoma are not known. There is no doubt that fair skin and red hair impose an important risk for the development of melanoma, but this and other studies have shown that these pigmentary characteristics exert their effect on melanoma risk independently of the presence of MC1R variant alleles. On one hand, carriers of MC1R variant alleles who have fair skin and/or red hair have a much higher melanoma risk than carriers of the same MC1R variant alleles who have darker skin and/or different hair colors. On the other hand, persons with a fair skin type have a much higher risk of melanoma if they also carry MC1R variant alleles.

An important function of the MC1 receptor obviously is influencing pigment metabolism through controlling the switch from pheomelanin to eumelanin and hence by determining the ability to protect against ultraviolet radiation (Healy *et al*, 2000). To explain the association between *MC1R* gene variants and melanoma, however, other functions of the MC1 receptor must play an important part. Besides an influence on skin melanogenesis, α-MSH, via the MC1 receptor, also influences proliferation and differentiation of keratinocytes and melanocytes and thus may affect growth and development of melanoma cells (Lunec *et al*, 1990; De Luca *et al*, 1993; Abdel-Malek *et al*, 1995; Chakraborty *et al*, 1996; Suzuki *et al*, 1996; Valverde *et al*, 1996). Furthermore, α-MSH can exhibit immunomodulatory and anti-inflammatory activities via its receptor (Wintzen and Gilchrest, 1996; Hedley *et al*, 1998; Morandini *et al*, 1998; Luger *et al*, 1999; Haycock *et al*, 2000). It is conceivable that the increased risk of cutaneous melanoma in individuals carrying *MC1R* gene variants is modulated by the effects of complete or partial inactivation of the MC1 receptor, which may modulate the effects of α-MSH on proliferation and differentiation of different cells or by hampering the immune defense against genetically changed melanocytes.

In conclusion, the presence of *MC1R* gene variants appears to play an important part in the pathogenesis of cutaneous melanoma, although the exact mechanism underlying this increased risk remains to be determined.

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