

activation and consequent production and deposition of mucinous material in the dermis.

As regards therapy, LM is very difficult to treat. Various agents have been tried, including melphalan, prednisone, cyclophosphamide, clorambucil, radiation, PUVA, plasma exchange, 2-chlorodeoxyadenosine, isotretinoin, photophoresis and interferon- α . None of these agents has been universally effective and many are associated with significant toxicity. Our patient's clinical state dictated an exclusively topical therapy using steroids and emollient creams, which produced a moderate improvement.

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The Asp84Glu Variant of the MC1R Gene in Norwegian Melanoma Patients

Sir,

Pigmentation traits are dependent on the relative amounts of eumelanin and pheomelanin present in the skin. Eumelanin is photoprotective whereas the red pheomelanin may contribute to UV-induced skin damage, caused by free radicals generated after UV exposure. In mammals, the relative proportions of pheomelanin and eumelanin are regulated by melanocyte-stimulating hormone (MSH), which acts via its receptor, MC1R (MIM 155555), on melanocytes (1, 2).

Variants of MC1R were associated with sun sensitivity and red hair in a UK population (2), and were considered a susceptibility gene for melanoma development. The Asp84Glu variant was later found to be associated with sporadic melanoma independent of skin type (3).

In this study we wanted to analyze this specific mutation in Norwegian melanoma patients, given the reported high frequency of Asp84Glu in melanomas.

MATERIAL AND METHODS

Patients

DNA from patients participating in the Norwegian Melanoma Project 1991–93 was extracted from paraffin-embedded lesions (for details, see protocols in QIAamp Blood Kit and QIAamp Tissue Kit Handbook 01/97). Specimens were collected from 2 hospitals in Oslo. The lesions were 69 melanomas, 8 atypical naevi and 20 benign naevi. All patients were Caucasian, and the skin type was reported in 63/69 patients with melanoma. The majority of patients were Fitzpatrick skin types II (22/63) and III (32/63). Eight of 64 melanoma patients were red-haired and 18/63 had freckled skin.

Mutation analysis

The MC1R gene was amplified by nested PCR using primers 5'-ACAGGACTATGGCTGTGC-3' and 5'-GCGTAGAAGATGGAGATG-3' to amplify a 503 bp product. The next PCR used 5'-TGA GCTTGGTGGAGAACGC-3' and 5'-AGGAAGCAGAGGCTGGA CAG-3' to produce a 253 bp product using the 503 bp product as template.

PCR conditions were 95°C for 3 min, followed by 30 cycles at 94°C (30 s), 54°C first reaction/60°C second reaction (30 s), 72°C (30 s). The Asp84Glu allele was identified by digestion with AVA II (37°C, 18 h), and the products were examined after electrophoresis in 2% agarose gel. Wild type (asp/asp) was identified by 153 and 100 bp fragments and homozygotes for the mutant allele (glu/glu) by 253 bp fragments.

Mutants were analyzed by automated sequencing using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The products were prepared for sequencing using the PCR product pre-sequencing kit (Amersham Life Science, Cleveland, OH) according to the manufacturer's instructions.

RESULTS

One variant allele (1/69) was found in heterozygous form at codon 84 in 1 melanoma (Fig. 1). The finding was confirmed by sequencing (Fig. 2). No naevi (0/28) harbored variant alleles at codon 84.

DISCUSSION

Our study did not support the view that the Asp84Glu variant is associated with melanoma, as reported by the case-control study of Valverde et al. (3).

In a larger case-control study by Ichii-Jones et al. (4), allele frequencies did not differ between melanoma cases and controls. They studied the Asp84Glu variant and 2 other variant alleles thought to be relatively common (2). Recently, Healy et al. (5) reported, based on new investigations, that the Asp84Glu variant is not as frequent as previously suspected in melanoma patients.

To date, more than 20 variants of MC1R are known. Three particular variants, Arg151Cys, Arg160Trp and Asp294Cys, are strongly associated with red hair, with a relative risk of 8–15. These same 3 variants were over-represented in individuals with fair skin (6).

There seems to be an agreement that MC1R variants are associated with red hair and fair skin, phenotypes that render

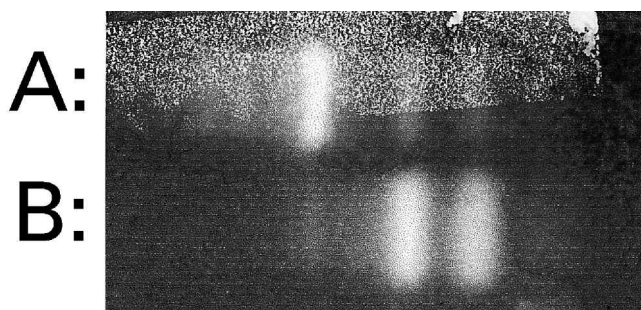


Fig. 1. PCR products after nested PCR and digestion by *AVA* II. Lane A: heterozygous mutant; lane B: wild type.

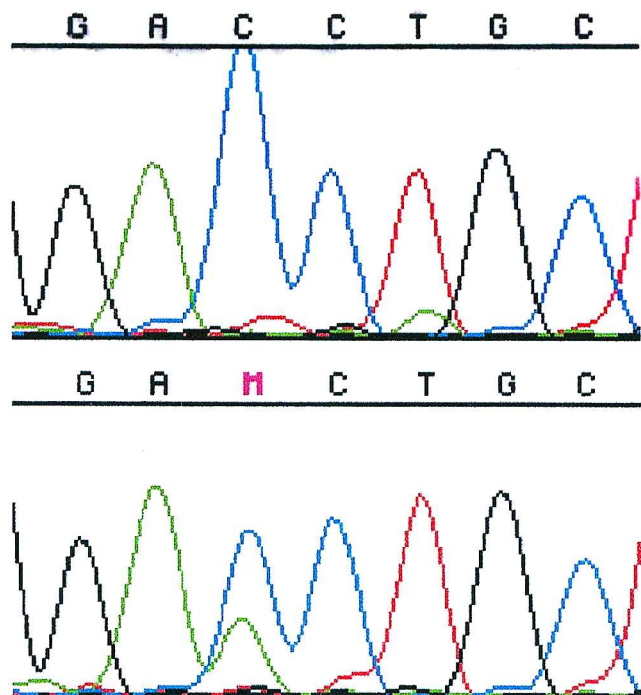


Fig. 2. Sequences showing wild-type *asp/asp* (top) and heterozygous mutant *asp/glu* (bottom).

individuals susceptible to cutaneous melanoma. Whether specific variants act independently of skin type has still not been elucidated. A recent large Australian study (7) demonstrated a doubled risk of cutaneous melanoma for each of the 3 above-mentioned alleles, a risk that disappeared when studying pale-skinned individuals alone. The risk persisted in

persons with a medium-to-dark complexion. The authors concluded that the effect of *MC1R* variant alleles on cutaneous malignant melanoma is partly mediated via determination of pigmentation phenotype. Interestingly, these alleles may negate the protection normally afforded by darker skin colouring.

We conclude that the originally reported association between a specific *MC1R* allele, *Asp84Glu*, and melanoma is rare in a Norwegian population. The literature shows that *MC1R* variant alleles are more frequent amongst melanoma patients, but it is still unclear whether variants act independently of skin type.

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