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Val92Met variant of the melanocyte stimulating hormone receptor gene

Sir — Recently Valverde *et al.*[1] reported in *Nature Genetics* variants of the human melanocyte stimulating hormone receptor (MSHR) gene[2] and their association with different shades of hair colour. One of the most frequent mutations described was Val92Met, present in individuals with light and deep red hair as well as in individuals with skin type I (always burn, never tan) and type II (always burn then slight tan). This mutation was thought to alter the α-helix structure of the second transmembrane domain of the MSHR, but no conclusion was drawn in the absence of functional studies. We have also found the Val92Met variant of MSHR in individuals with skin type I (present in 7/11 cases examined, 63%). When we expressed the Val92Met variant of MSHR in COS-1 cells, we found that the endogenous hormone α-melanocyte stimulating hormone (αMSH) had approximately five times lower potency in displacing a radiolabelled analogue of α-MSH as compared to the wild-type receptor (Fig. 1). In mammals the relative amounts of eumelanin (black pigment) and phaeomelanin (red pigment) are regulated by action of αMSH on its receptor; the higher the affinity of αMSH to its receptor the greater the eumelanin level. Eumelanin renders protection, whereas phaeomelanin generates free radicals in response to UV radiation and thus causes damage in type I and II skin. The loss in potency of αMSH due to Val92Met mutation may account for the decreased synthesis of eumelanin resulting in higher levels of phaeomelanin and thus explains red hair as well as burning of type I and II skin.

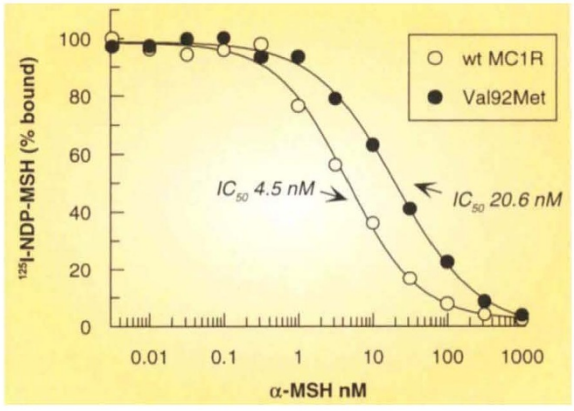

Fig. 1 Displacement of radiolabelled analogue of αMSH by MSH from wild-type and Val92Met variant of the MSH-R expressed in COS-1 cells. Primers corresponding to the 5' untranslated region (gtggggacctggaggcctcca) and 3' untranslated region (aggaactgcccagggtcacac) of the *MSHR* gene[2] were used to perform PCR on genomic DNA prepared from white blood cells of the type I skin individuals. The PCR was done in a final volume of 50 µl and contained 1 µg genomic DNA, 1× pfu DNA polymerase buffer, 200 µM of each deoxynucleotide, 1 µM of each primer and 1 U of pfu DNA polymerase (Stratagene). The PCR thermal profile used was 93 °C for 45 s, 60 °C for 20 s and 72 °C for 60 s for a total of 30 cycles. Resulting PCR products containing the entire coding region of *MSHR* were cloned in PCR 3 vector (InVitrogen) and sequenced using the Sequenase kit (Amersham). DNAs from the Val92Met variant as well as wild-type *MSHR* were used to transfect COS-1 cells using lipofectin as described[2]. Cells were harvested 60 h post-transfection, washed once in binding buffer (minimum essential medium with Earle's salts, 25 mM HEPES pH 7.0, 0.2% bovine serum albumin, 1 mM 1,10-phenanthroline, 0.5 mg/litre leupeptine and 200 mg/litre bacitracin) and distributed in 96-well plates. Cells were then incubated at room temperature for 2 h with 0.05 ml of binding buffer containing 15,000 cpm of [^{125}I]-labelled analogue of αMSH and the appropriate concentration of unlabelled α-MSH. Plates were then put on ice, cells washed with 0.1 ml of ice cold binding buffer, and detached from plates with 0.1 ml of 0.1N NaOH. Radioactivity was counted and data analysed by an iterative, non-linear curve fitting programme called GraFit. IC_{50} values are the mean of three separate experiments.

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