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Genetic screening of 15 SNPs in the *MC1R* gene in relation to hair colour in Danes

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Abstract. The Melanocortin 1 Receptor (MC1R) is an important regulator of pigmentation in hair and skin. *MC1R* mutations D84C, R151C, R160W, D294H have been designated strong red hair alleles (R) and V60L, V92M, R163Q as weak red hair alleles (r). We have developed a package of 15 SNPs from the *MC1R* gene and typed 34 Danes with red hair and 56 Danes with blond and dark hair, 65 of the individuals were related in 8 families. In our model, we designated R142H, 29InsA and 179InsC as R alleles. Only red-haired individuals were homozygous for R or compound heterozygous for two R alleles, and the wt/wt genotype was observed only in samples from individuals with blond or dark hair.

Keywords: SNP; MALDI-TOF; MC1R

1. Introduction

Hair, eye and skin pigmentation in humans is a result of the synthesis and the deposition of melanin. The Melanocortin 1 Receptor (MC1R) is an important regulator of melanin synthesis and numerous mutations in the single-exon *MC1R* gene encoding MC1R have been reported. Some of these mutations affected the function of MC1R and they were found in high frequencies in individuals with red hair. Certain MC1R mutations have been designated as strong "R" (D84C, R151C, R160W, D294H) or weak "r" (V60L, V92M, R163Q) red hair alleles [1]. In addition, these mutations have been shown to affect other physical traits such as skin colour, freckling and mole count [1,2]. The purpose of this study was, first to design and validate a package of MC1R SNPs, and secondly, to investigate the MC1R SNPs in relation to hair colour.

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2. Materials and methods

Eight families with a total of 65 Danish, Caucasian individuals were selected from the Copenhagen family bank (http://rclink.imbg.ku.dk/rclink.htm.). In addition, samples were collected from 25 unrelated volunteers. PCR was performed on chelex extracted DNA or on preserved lymphocytes on FTA-paper. Five short fragments covering 793 bp of the *MC1R* gene were amplified in a multiplex PCR. Alternatively, a 1648 bp fragment covering the entire coding region, 626 bp of the promoter and 59 bp downstream of the coding region was amplified with the purpose of determining haplotypes that could not be deduced by family studies. Single base extension (SBE) primers were designed to detect 15 SNPs from the *MC1R* gene: Eight missense mutations (V60L, D84C, V92M, R142H, R151C, R160W, R163Q, D294H), two insertion mutations (179InsC, 29InsA), two silent mutations (P300P, T314T) and three SNPs near the important regulatory element, SP-1, in the *MC1R* promoter (rs3212359, rs3212360, rs3212361). PCR amplification conditions were as previously described [3]. The 15 MC1R SNPs were typed in a multiplexed SBE reaction using either SNaPshot reaction chemistry and detection by capillary electrophoresis [3] or biotin-ddNTPs with the monomeric avidin triethylamine purification (MATP) protocol and detection by MALDI-TOF MS [4].

3. Results

Fig. 1 shows the results of SNP typing a sample from person with red hair using multiplex PCR, multiplex SBE and MALDI-TOF MS. The spectrum has 22 peaks representing 3 internal standards, 4 heterozygous loci and 11 homozygous loci. Two R alleles were detected (R160W and R151C). By typing the parents, it was concluded, that R160W was inherited from the mother and that the R151C was inherited from the father. Thus, the two R alleles were located on different chromosomes (compound heterozygous). We typed 34 individuals classified as red-haired and 56 classified as dark or blond-haired. Three alleles R142H, 29InsA and 179InsC were designated as R alleles in our model. The genotype R/R was found in 18 red-haired individuals and not in individuals with dark

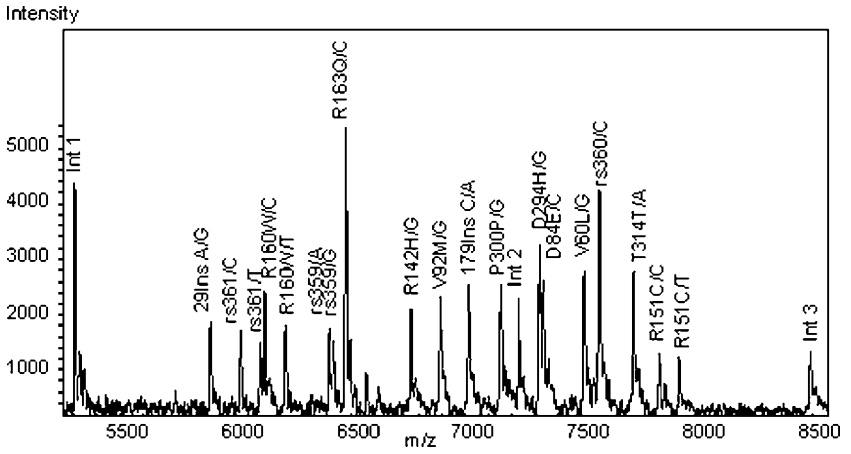

Fig. 1. MALDI-TOF spectrum of 15 MC1R SNPs typed in a multiplex SBE reaction from a person with red hair. Int 1, Int 2, and Int 3 are internal size standards.

Table 1
Haplotypes and number of observations in parentheses found in Danish families with red-haired members

SNP	H1 (29)	H2 (16)	H3 (29)	H4 (20)	H5 (3)	H6 (9)	H7 (2)	H8 (16)	H9 (2)	H10 (2)	H11 (2)
rs359	A	G	G	A	G	A	A	G	A	A	G
rs360	C	C	C	C	C	C	C	C	C	C	C
rs361	T	C	C	T	C	T	T	C	C	C	C
29InsA	G	G	G	G	G	T	G	G	G	G	G
V60L	G	G	G	G	G	G	G	T	G	G	G
D84C	C	C	C	C	C	C	C	C	C	C	C
V92M	G	G	G	G	G	G	G	G	G	G	A
R142H	G	G	G	G	A	G	G	G	G	G	G
R151C	C	C	T	C	C	C	C	C	C	C	C
R160W	C	C	C	T	C	C	C	C	C	C	C
R163Q	C	C	C	C	C	C	T	C	C	C	C
179InsC	A	A	A	A	A	A	A	A	C	A	A
D294H	G	G	G	G	G	G	G	G	G	G	G
P300P	G	G	G	G	G	G	G	G	G	G	G
T314T	A	A	A	A	A	A	A	A	A	G	G

and blond hair. Thirteen individuals were compound heterozygous and three were homozygous for R151C. The remaining 14 red-haired individuals had the genotype R/r or R/wt. Wt/wt was observed in 11 individuals who all had dark or blond hair. MC1R SNP haplotypes were determined from the genotyped families (Table 1). In 16 of the 18 red-haired individuals genotyped as R/R, it was possible to deduce that the two R mutations were located on different chromosomes. We did not have samples from the parents of the two red-haired individuals genotyped R/R.

4. Discussion

A sample genotyped as R/R strongly indicate the sample originates from a person with red hair, whereas wt/wt indicates that the sample originate from a person with blond or dark hair. It is not possible to determine the haplotypes of an individual without additionally testing. However, haplotypes may be important for the phenotype, e.g. RR/wt may be dark or blond and not red-haired. The two highly polymorphic SNPs r359 or r361 may be useful to determine the haplotype using allele-specific PCR or allele-specific hybridisation.

The MC1R package may be used to select red-haired individuals that do not have the R/R genotype. These individuals may be used in search for new interesting MC1R alleles. Furthermore, families with red-haired individuals that do not have the R/R genotype may be used for linkage studies in the search for new genes involved in the regulation of melanin synthesis.

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