International Congress Series 1288 (2006) 816–818

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# Determination of forensically relevant SNPs in the MC1R gene

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**Abstract.** Genetic prediction of physical appearance is a very attractive prospect for forensic investigations. The association between some genetic variants of the MC1R gene and red hair colour has been already proved. Our study focuses on the potential forensic applicability of variation within this pigmentary gene. The obtained results indicate that a major role in determination of red hair colour in our region is played by the diminished-function MC1R variants—R151C, R160W. The relatively high frequency of these variants and their significant association with red hair makes analysis of these particular positions very interesting from the perspective of phenotype prediction.


*Keywords:* Physical traits; Red hair colour; Genetic prediction; MC1R

## 1. Introduction

The association between genetic variants and particular phenotypic features has been extensively explored in various scientific disciplines [1]. The great variability in hair and skin colour among humans means that genetic prediction of these physical traits is an attractive prospect for forensic investigations. Genetic typing of biological traces collected at scenes of crime could be a source of valuable information about a donor's physical characteristics. The ratio of two pigment types: brown or black eumelanin and red or yellow pheomelanin is responsible for a great amount of variation in hair and skin colour. The melanocortin 1 receptor gene (MC1R) plays a key role in the eumelanin/pheomelanin ratio in humans and hence its influence on hair and skin colour is crucial [2]. Some allelic

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0531-5131/ © 2005 Published by Elsevier B.V.
doi:10.1016/j.ics.2005.08.021

variants of the MC1R are significantly associated with the overproduction of pheomelanin, which at least in some populations manifests itself in red hair and light skin. Variation within the MC1R gene is especially high among Europeans and has a significant impact on pigmentary phenotype in this ethnic group [3]. Our goal was to check the variation within the MC1R gene characteristic for our region and evaluate the usefulness of its analysis for forensic investigations.

## 2. Materials and methods

The project is being carried out in cooperation with the Department of Dermatology at the Jagiellonian University in Cracow. The donors' physical characteristics were examined by a single dermatologist, who also collected buccal swabs for the purpose of genetic analysis. The collected material was subjected to DNA isolation using the standard organic method and phenol extraction. DNA concentration was measured with the fluorimetric method. The complete MC1R gene was amplified using primers described by Kanetsky et al. [4]. PCR products were purified using Microcon 100 columns (Millipore) and subjected to cycle sequencing using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Products of sequencing reactions were subjected to analysis using genetic analyser ABI 3100. Comparison of allele frequencies between the red headed group and the control group was done with Pearson's $\chi^2$ test. For the purpose of examination of forensic specimens which are often subjected to degradation, we designed multiplex amplification of MC1R fragments covering the variable positions associated with the red hair phenotype (R151C, R160W, D294H and additionally V60L, D84E, V92M, R163Q). The best amplification results were obtained with the Qiagen Multiplex PCR Kit. Products of amplification were subjected to minisequencing using a SnaPshot Multiplex Kit (Applied Biosystems) and extension primers optimised for the purpose of this study.

## 3. Results

Up till now we have obtained complete MC1R sequence data for 35 red haired individuals and 108 controls with different hair colour. The obtained results show that MC1R variation in the studied Polish population sample is high and is similar to the level of variation characteristic of other populations of northern European origin [2,5]. Our study revealed 19 variable sites of which 14 are non-synonymous changes (Table 1). The sequence data determined for the MC1R gene proved that the diminished-function variants–R151C, R160W–play a significant role in determination of red hair colour in our region. The single homozygote R151C ascertained during the study and all 4 homozygotes R160W as well as 10 of 11 heterozygous variants R151C, R160W revealed red hair. Prediction of the red hair phenotype in the case of these genotypes is strong with 94% of individuals having red hair and pale skin. The above variants appear in red-haired individuals in combination with some other polymorphic sites, and finally 30 out of 35 red headed individuals had at least one of these polymorphisms. The high frequency in the population of variants R151C, R160W and their very high association with red hair ($p \leq 0.001$) makes analysis of these particular positions very interesting for the purpose of phenotype prediction. Our study disclosed only three D294H diminished function variant alleles ($f = 1.1\%$), while the penetration of this allele in other European populations

Table 1
MC1R allele frequencies in 34 unrelated red-haired individuals and a group of 108 controls

| Nucleotide change | Amino acid change | Red hair, 68 alleles [%] | Controls, 216 alleles [%] | Significant differences |
|---|---|---|---|---|
| Consensus | | 5.9 | 51.9 | $p \leq 0.001$ |
| G178T | V60L | 2.9 | 8.3 | – |
| C252A | D84E | 1.5 | 0 | – |
| G274A | V92M | 7.4 | 9.7 | – |
| C325T | R109W | 0 | 0.5 | – |
| G425A | R142H | 4.4 | 0.5 | $p \leq 0.025$ |
| C444T | Y148Y | 0 | 0.5 | – |
| C451T | R151C | 27.9 | 4.6 | $p \leq 0.001$ |
| C456A | Y152OCH | 2.9 | 0 | – |
| T464C | I155T | 2.9 | 0.9 | – |
| C478T | R160W | 35.3 | 9.3 | $p \leq 0.001$ |
| G488A | R163Q | 1.5 | 4.2 | – |
| C561T | A187A | 0 | 0.5 | – |
| G652A | A218T | 0 | 0.5 | – |
| G699A | Q233Q | 0 | 0.5 | – |
| C766T | P256S | 1.5 | 0 | – |
| A832G | K278E | 0 | 0.5 | – |
| G880C | D294H | 4.4 | 0 | $p \leq 0.01$ |
| A942G | T314T | 8.8 | 9.7 | – |
| C948T | S316S | 0 | 0.5 | – |

exceeds 3%. All individuals who had this variant have red hair. Thirty of the analysed red-haired individuals carry more than one allele variant. Three individuals (all blonde-red) are simple heterozygotes with one consensus allele. One individual with blonde-red hair has two consensus alleles. The developed SNaPshot based assay provides selective analysis of variable sites within the MC1R gene, which have a significant correlation with red hair. These positions are amplified in a triplex reaction and performed experiments proved that the multiplex test also enables reliable and sensitive determination of MC1R variants in typical forensic specimens such as blood or semen stains and bone samples. Analysis of additional variation present in other genes will give a more complete picture of the genetics of human pigmentation and create an opportunity for future tests that will constitute a useful supplement that can be utilised in forensic sciences.

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