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Melanocortin-1 receptor gene variants in four Chinese ethnic populations

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ABSTRACT

There is strong relationship between melanocortin-1 receptor (MC1R) gene variants and human hair color and skin type. Based on a sequencing study of MC1R gene in 50 individuals from the Uygur, Tibetan, Wa and Dai ethnic populations, we discuss the occurrence of 7 mc1r variants consisting of 5 nonsynonymous sites (Val60Leu, Arg67Gln, Val92Met, Arg163Gln and Ala299Val) and 2 synonymous sites (C414T and A942G), among which C414T and Ala299Val were reported for the first time. Confirmation and analysis were also made of 122 individuals at three common point mutations (Val92Met, Arg163Gln, A942G) using PCR-SSCP. The frequency of Arg163Gln variant varies in the four ethnic populations, with percentage of 40%, 85.0%, 66.2% and 72.7%, respectively, while those of Val92Met and A942G are roughly similar in these four populations. The different environments, migration and admixture of various ethnic groups in China might have impact on the observed frequency of Arg163Gln.

Key words: *MC1R gene, ethnic populations, nonsynonymous site, synonymous site.*

INTRODUCTION

The variation in human hair and skin color in different geographic regions of the world is the result of differences in two principal forms of melanin, the red-yellow phaeomelanins and the black-brown eumelanins, which are present in the epidermal layer of human skin and hair[1],[2]. The type of melanin produced is under the control of two genes, identified initially by the mouse mutation, extension and agouti. The extension gene is expressed in melanocytes, producing the melanocyte stimulating hormone receptor (MSHR) or melanocortin-1 receptor (MC1R)[3],[4]. The human MC1R gene, homologous to the mouse extension locus, was cloned[3],[5],[6], located to chromosome 16q24[7] and shown to encode the MC1R protein. Expressed on cutaneous melanocytes[3], MC1R is a seven transmembrane domain G protein-coupled receptor of 317 amino acids belonging to the melanocotin receptor subfamily and has high binding affinity for MSH and ACTH[8, 9]. In addition, some other studies show that MC1R variants are associated with the coat colors in cattle [10],[11], fox[12], and horse[13].

Studies of MC1R polymorphism have been made in European, African and Asian populations. Valverde et al[14], Box et al[15] and Smith et al [16] reported 18 variants of MC1R in red hair/fair skin individuals. In a recent study by Rana et al [4], Africans were reported to be lack of variation while six variants were found in Asian populations. However, little is known about the variants of MC1R gene in Chinese populations, let alone data in Chinese ethnic populations. In this paper we examined the polymorphism of the human MC1R gene in four Chinese ethnic populations.

SAMPLES AND METHODS

Human samples

A total of 122 individuals from 4 Chinese ethnic populations (35 Uygur from Xinjiang Province, 20 Tibetans from Qinghai

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Province, 34 Was and 33 Dais from Yunnan Province.) volunteered as samples for the study. Genetically, none of them was known to be related to any other volunteer and all of their parents and grandparents were descendants of the mentioned nationality.

Amplification and sequencing

Genomic DNA from blood was amplified by PCR to obtain the entire coding region of human MC1R gene according to Rana et al[4]. PCR products were purified with gel extraction kits (Watson BioMedicals, Inc.) and sequenced with an Applied Biosystem ABI 377 sequencer (PE Biosystems) using BigDye^{TM} Terminator Cycle Sequencing Kit (Perkin-Elmer) under the manufacturer's instructions.

Identification of variants

Both sequencing and SSCP (single-strand conformation polymorphism) analysis were used to identify the MC1R variants. In our preliminary study, 10 Dais, 15 Tibetans, 15 Uygurs and 10 Was were sampled randomly for sequencing. Three variants (Val92Met, Arg163Gln and A942G) were chosen for PCR-SSCP analysis and gene frequency calculation in 122 individuals. PCR-SSCP was performed with three sets of primers to yield three 200-300bp products using the method in reference[17].

Calculation of allele frequency

The mathematical equations of the allele frequencies and the genotype are given by:

$p=(2NAA+NAa)/2N$ $q=(2Naa+NAa)/2N$.

In which p and q are the allele frequencies of A and a; NAA, NAa and Naa are the numbers of AA (wild-genotype), Aa (heterozygous variant-genotype) and aa (homozygous variant-genotype).

RESULTS

MC1R variants

The entire coding sequence of the MC1R gene was sequenced in 50 individuals from the Urgur, Tibeten, Dai, and Wa nationalities. Compared to the published sequences[3-6], [18], sequences of our samples differed from the human consensus sequence at five nonsynonymous sites (at codon 60, 67, 92, 163 and 299) and at two synonymous sites (at nucleotide 414 and 942) (Tab 1). In the previous study of MC1R variants, Val92Met and Val60Leu were reported to be frequent in the red hair/fair skin samples[14]. In this study, heterozygous Val60Leu was found only in one Uygur individual; whereas the Val92Met variant was found in Uygur, Dai, Wa ethnic populations, but no homozygote in Tibetan. Furthermore, the Val92Met variant always went with the A942G variant in our samples.

Tab 1. Variants and their regions located in humanMC1R detected in four ethnic population

Region of human MC1R	Variants	
First transmembrane region	Val60Leu	(G178T)
First intracellular region	Arg67Gln	(G200A)
Second transmembrane region	Val92 Met	(G274A)
Third transmembrane region	Ile138Ile	(C414T)
Second intracellular region	Arg163Gln	(G488A)
Seventh transmembrane region	Ala299Val	(C896T)
Fourth intracellular region	Thr314Thr	(A942G)

The variants are indicated by an amino acid and nucleotide change, as well as the codon and nucleotide position compared with the human consensus sequence.

Tab 2. Variant frequencies within four ethnic groups

Ethnic			Genotype[a]			Allele frequencyb	
group	n	Variant allele	AA	Aa	aa	p	q
Tibetan	20	Val92Met	16	4	0	0.9000	0.1000
		Arg163Gln	1	4	15	0.1500	0.8500
		A942	16	4	0	0.9000	0.1000
Uygur	35	Val92Met	29	4	2	0.8857	0.1143
		Arg163Gln	14	14	7	0.6000	0.4000
		A942G	27	6	2	0.8571	0.1429
Dai	33	Val92Met	15	15	3	0.6818	0.3182
		Arg163Gln	6	6	21	0.2728	0.7272
		A942G	15	15	3	0.6818	0.3182
Wa	34	Val92Met	27	6	1	0.8824	0.1176
		Arg163Gln	5	13	16	0.3382	0.6618
		A942G	26	7	1	0.8676	0.1324

[a]he number of individual alleles genotype: AA=homozy- gous wild-type, Aa=heterozygous variant, aa=homozygous variant.

[b]p represents the frequency of the A allele; q represents the frequency of the a allele?

Rana et al[4] reported the Arg163Gln variant to be associated with the East and Southeast Asian populations. In this study, a very common Arg163Gln variant was also found in the four ethnic groups concerned, including 21 of 35 Uygurs, 19 of 20 Tibetans, 29 of 34 Was and 27 of 33 Dais. The Arg67Gln/Arg163 variant in one Dai individual was also observed in other East and Southeast Asian populations (Rana et al, 1999), which is a combination of the Arg163Gln variant. Besides,

one synonymous mutation and one nonsynonymous mutation were first found in Uygur (C414T and Ala299Val).

Gene frequency

The PCR-SSCP analysis was used to genotype the three variants, Val92Met, Arg163Gln and

A942G in 122 individuals. The gene frequency of the Arg163Gln variant was found to be significantly different in the four ethnic groups, with the highest (85.0%) in Tibetan, the lowest (40%) in Uygur, and the intermediate in Dai (72.7%) and Wa (66. 2%). The gene frequency of the Val92Met differed in the Dai (31. 8%), the Tibetans (10%), the Wa (11.8%) and the Uygur (11.4%). The A942G and Val92Met variant gene frequency for each of the four ethnic groups remained roughly similar, as listed in Tab 2. Hardy-Weinberg equilibrium was not rejected in all these ethnic groups (Data not shown).

DISCUSSION

MC1R is a regulator of eumelanin and phaeomelanin production, and its mutations might cause the changes in human hair and skin color[1], [2].

Three alleles (Arg151Cys, Arg160Trp and Asp294His) that are associated with red hair/fair skin phenotype have been reported in European individuals[5],[16]. Recently, Franderberg et al[19] found new evidence that the Arg151Cys mutation of MC1R can cause the synthesis of the red pigment. This evidence explains why the red hair person carries the Arg151Cys mutation. The Arg163Gln variant is present with relatively high frequency in the East and Southeast Asian populations[4],[20]. In consistent with those reports, our result shows a very common Arg163Gln variant in the four ethnic groups. It might suggest that the Arg163Gln polymorphism is associated with phaemomelanin-rich skin. But further functional study is required to confirm our expectation.

The Arg163Gln variant is found in American Indians as well as in East and Southeast Asian populations, while the allele appears at a very low frequency or even disappears in both Europeans and Africans. Rana et al[4] considered that the allele has increased rapidly in frequency in East Asians by positive Darwinian selection. We suggest that the random genetic drift, migration and the admixture of various ethnic groups might have impact on the frequency of the Arg163Gln variant in different populations. Firstly, the highest frequency and the most homozygous state in Tibetans might arise from genetic drift and little possibility of gene flows among different ethnic groups. The positive Darwinian selection is also a possible explanation. Secondly, the lowest frequency in Uygurs might be the result of their genetic admixture with Caucasians. This assumption can be further supported by results from other reports[17],[22]. On the other hand, considering the genetic admixture, it is explicable that an European specific allele, Val60Leu, is present in one Uygur individual. Lastly, the similar frequencies in the Dai and the Wa might be explained by their similar geographic locations and living environments.

Tab 2 shows that the gene frequency of A942G in the Dai and the Wa originating in southern China is 20% and the frequency in the Tibetan and the Uygur is 10% and 12%, respectively; whereas Rana et al[4] reported a 42% gene frequency in the Africans, 23% in east and southeast Asians and the absence in American Indians. It seems that the gene frequency of A942G decreases with increasing latitude. Nevertheless, more data are needed to examine this possibility.

In addition to the three variants (Val92Met, Arg163Gln and A942G), two new mutations are found in two Uygur individuals, one with C414T in homozygous state and the other with Ala299Val in heterozygous state. The occurrence of two new mutations and the question whether the differences can suggest the genetic divergences in these four ethnic groups or not, require further investigation.

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