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Distribution of Two Asian-Related Coding SNPs in the *MC1R* and *OCA2* Genes

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Abstract Very little is known about the genes and mechanisms affecting skin lightening in Asian populations. In this study, two coding SNPs, c.G1129A (R163Q) at the *MC1R* (melanocortin 1 receptor) gene and c.A1962G (H615R) at the *OCA2* (oculocutaneous albinism type II) gene, were investigated in a total of 1,809 individuals in 16 populations from various areas. The Q163 and R615 alleles prevailed almost exclusively in East and Southeast Asian populations. Wright's F_{ST} was 0.445 for R163Q and 0.385 for H615R among the 16 populations. The frequency of the Q163 allele was higher in Northeast Asians than in Southeast Asians. The frequency of the R615 allele was highest in South China and unlikely to be associated with levels of ultraviolet radiation. This allele may be a good marker to study the genetic affinity among East Asians because of its restricted distribution and marked difference in allele frequency.

Keywords *MC1R* · *OCA2* · Pigmentation · Polymorphism · Population study · Skin color

Introduction

Skin color, one of the most visible signs of human biological variation, is markedly diverse among populations. Skin color shows 88% of the total variation among regions in contrast to classical genetic markers and DNA polymorphisms. The global variation in skin color is strongly suggested to have been affected by natural selection (Relethford 2002). Skin color among indigenous peoples is correlated with levels of ultraviolet radiation and becomes lighter in more northerly latitudes (Jablonski and Chaplin 2000). In spite of many candidate genes, relatively little is understood about the genetic and molecular basis of human skin variation. Recently, L374F in the membrane-associated transporter protein (*SLC45A2* gene) and A111T in a putative cation exchanger protein (*SLC24A5* gene) were found to have a unique distribution in allele frequency: the mutant alleles prevail extensively in Europeans, whereas the wild-type alleles are fixed in Africans and Asians. These two mutant alleles show strong evidence of having undergone positive selection in Europeans (Graf et al. 2005; Lamason et al. 2005; Nakayama et al. 2002; Soejima et al. 2006; Yuasa et al. 2004, 2006). Asian populations also have light skin, for which no genes are known to be responsible, although several candidate genes have been suggested (McEvoy et al. 2006; Myles et al. 2007).

Melanocortin 1 receptor (MC1R), belonging to a family of G protein-coupled receptors, leads to the production of eumelamin after the binding of α-melanocyte-stimulating hormone (α-MSH). Some mutations in the *MC1R* gene on chromosome 16q24.3 are associated with red hair, fair skin, and freckles (Valverde et al. 1995). The R163Q (CGA→CAA, refSNP ID: rs885479) variant is observed at high frequencies in East and Southeast Asians and Native Americans (Makova and Norton 2005; Nakayama et al. 2006; Rana et al. 1999). Although the Q163 allele had slightly less affinity for α-MSH in binding and second-messenger studies, this mutation was not considered to have an important role in supporting the red-haired phenotype (Ringholm et al. 2004). The *OCA2* gene (P-related gene) on chromosome

15q11.2–q12, the human homolog of the murine pink-eyed dilution (p) gene, plays a role in regulating the pH of melanosomes. Some mutations in the *OCA2* gene result in oculocutaneous albinism type II (Oetting et al. 2005; Puri et al. 2000). The H615R (CAT→CGT, refSNP ID: rs1800414) mutation at the *OCA2* locus is suggested to be Asian-specific (Lee et al. 1995). However, there have been few studies of the H615R mutation. We therefore investigated the frequency of the H615R polymorphism in several populations as well as that of the R163Q polymorphism in the *MC1R* gene.

Materials and Methods

DNA samples

DNA samples were obtained from 1809 unrelated individuals, mainly living in various areas of Eurasia. The donors were selected at random, irrespective of skin color, and their phenotypic data were unavailable. All samples except two African population samples were the same set as used in our previous study (Yuasa et al. 2006). The African samples were collected in Germany.

Simultaneous Typing of the Two Coding SNPs

DNA samples were typed simultaneously for the two polymorphisms on the basis of the amplified product length polymorphism (APLP) method (Watanabe et al. 1997; Yuasa et al. 2004). A PCR cocktail consisted of 100 µl of Multiple PCR Master Mix in Multiplex PCR Kit (Qiagen), 92 µl of six primers with a concentration of 10 pmol/µl, and 8 µl of water. The nucleotide sequence and amount of each primer are shown in Table 1. PCR was performed in a volume of 8 µl containing 7.5 µl of the PCR cocktail and 0.5 µl of a solution containing about 20 ng of genomic DNA. Cycle conditions were 95°C for 15 min, then 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 40 s, and a final extension step of 10 min at 72°C, in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). The products were separated

Table 1 PCR primers for simultaneous typing of the *MC1R* R163Q and *OCA2* H615R polymorphisms and their amounts in 200 µl of PCR cocktail

Name	Sequence (5′→3′) [a]	Amount (µl)
MC1R-163F	aCTCCATCTTCTACGCACTGCG	16
MC1R-163RA	tttaGATGGCCGCAACaGCTT	20
MC1R-163RG	GATGGCCGCAACGaCTC	16
OCA2-615FG	attaCTGTGGTTTCTCTCTTACAtCG	16
OCA2-615FA	CTGTGGTTTCTCTCTTACtGCA	12
OCA2-615R	CATTTGGCGAGCAGAATCC	12

[a] Lowercase letters indicate noncomplementary nucleotides

using a polyacrylamide gel (9%T, 5%C), then visualized by ethidium bromide staining.

Statistical Analysis

Allele frequencies were estimated and Hardy–Weinberg equilibrium was tested using Arlequin program ver. 3.01 (Excoffier et al. 2005). Wright's F_{ST} statistic was used to estimate the proportion of variation attributable to differences in the R163Q and H615R mutations among 16 populations (Hartl and Clark 1997).

Results and Discussion

For the amplification of DNA fragments at a locus, the APLP method requires three primers: two allele-specific primers differing in length and one common primer on the opposite DNA chain. Figure 1 shows the banding patterns of PCR products obtained by simultaneous amplification of the two coding SNPs. Each genotype was clearly and unambiguously distinguished. The amplicons were generated only when the two allele-specific primers matched 3′ ends and the nucleotide substitutions were detected as amplified fragments with different lengths.

Table 2 summarizes differences in the distribution of the genotypes and allele frequencies for the *MC1R* and *OCA2* genes in a total of 1809 unrelated individuals from 16 populations in three regions. In each population, there was no statistically significant departure from Hardy–Weinberg equilibrium (data not shown).

Our data on the distribution of the Q163 allele at the *MC1R* locus were not inconsistent with those reported previously (Makova and Norton 2005; Nakayama et al. 2006; Rana et al. 1999). Wright's F_{ST} was estimated to be 0.445 among the 16 populations investigated in this study. The Q163 allele was observed at high frequencies in all eight Asian populations. The Japanese population in Okinawa had the highest frequency of 0.833 which was comparable to 0.85 in Ainu (Nakayama et al. 2006) and Tibet (Shi et al. 2001). The Q163 allele is very common in New World populations, which have an affinity with East Siberian and Northeast Asian populations (Harding et al. 2000; Rana et al. 1999). The frequency of this allele is

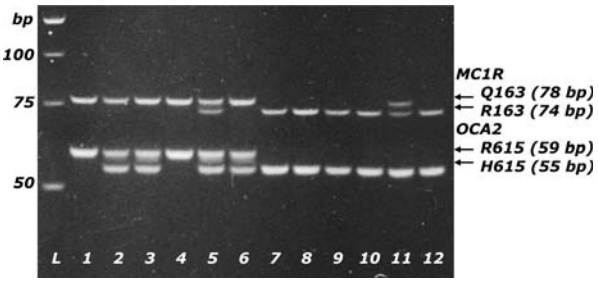

Fig. 1 Simultaneous genotyping of the R163Q and H615R polymorphisms. Lane L: 25-bp ladder. Lanes 1–6: Japanese. Lanes 7–12: Germans

Table 2 Distribution of two SNPs in the *MC1R* and *OCA2* genes

No.	Population	Collection place	*n*	*MC1R:* Genotypes of R163Q			*OCA2:* Genotypes of H615R			Allele frequency	
				R	R/Q	Q	H	H/R	R	*MC1R* Q163	*OCA2* R615
1	Nigerian	Northrhine-Westphalia	31	31	0	0	31	0	0	0.000	0.000
2	Ghanaian	Northrhine-Westphalia	36	36	0	0	36	0	0	0.000	0.000
3	German	Northrhine-Westphalia	199	188	10	1	198	1	0	0.030	0.003
4	German	Munich	92	79	12	1	92	0	0	0.076	0.000
5	French	Rheims	98	92	6	0	98	0	0	0.031	0.000
6	Turk	West Germany	200	172	26	2	194	6	0	0.075	0.015
7	Indian	New Delhi	107	96	10	1	107	0	0	0.056	0.000
8	Bangladeshi	Dhaka	118	88	29	1	116	2	0	0.131	0.009
9	Khalha	Ulaan Baator	173	13	72	88	109	57	7	0.717	0.205
10	Buryat	Eastern Mongolia	143	13	62	68	107	34	2	0.692	0.133
11	Japanese	Tottori	103	4	45	54	17	59	27	0.743	0.549
12	Japanese	Okinawa	87	3	23	61	24	44	19	0.833	0.471
13	Han Chinese	Shenyang	87	7	39	41	26	44	17	0.695	0.448
14	Han Chinese	Wuxi	119	19	50	50	19	64	36	0.630	0.571
15	Han Chinese	Huizhou	111	17	62	32	18	46	47	0.568	0.630
16	Indonesian	Surabaya	105	23	54	28	84	20	1	0.524	0.105
17	CEU[a]		60		no data		60	0	0	–	0.000
18	CHB[a]		45		no data		7	19	19	–	0.633
19	JPT[a]		44		no data		8	26	10	–	0.523
20	YRI[a]		60		no data		60	0	0	–	0.000
Wright's F_{ST}										0.445	0.385

[a] HapMap data, not included in calculation of Wright's F_{ST}

rather low in Han Chinese in Huizhou and Indonesians in Surabaya. A previous study of 30 Asian and Oceanian populations revealed that East Asians apparently had high frequencies (0.70 on average) in comparison with Southeast Asians (0.44) and Oceanians (0.01) (Nakayama et al. 2006).

The R615 allele at the *OCA2* locus was rare or not found in Indo-European and African populations, whereas it was very common in Asian populations. Wright's F_{ST} for the R615 allele was estimated to be 0.385 among the 16 populations and was lower than that for the Q163 allele at the *MC1R* locus. The R615 allele had a different distribution from the Q163 allele and was most common in Huizhou (0.63). Japanese had similar or somewhat low frequencies in comparison with Han Chinese in Wuxi and Huizhou. Khalhas, Buryats, and Indonesians in the surrounding areas showed the lowest frequencies among East Asian populations. Frequencies of the R615 allele in the populations from the International HapMap Consortium (http://www.hapmap.org/) are consistent with findings of the present study (see Table 2). The R615 allele is likely to have occurred more recently than the Q163 allele which was estimated to have arisen after the divergence of Asians, Europeans, and Africans and before the divergence of Asians and Native Americans (Rana et al. 1999) and 100,000–250,000 years ago (Harding et al. 2000). The R615 allele must have spread rapidly to Asian populations after its occurrence in China. Its distribution is not associated with ultraviolet radiation levels, and it is unlikely to contribute to the light skin of Mongolians and Buryats. The R615 allele may be a good marker to study the genetic affinity among East Asians because it is nearly restricted to them and its frequency is extremely different among them.

No genes are known to be responsible for light skin in Asian populations. Recently, McEvoy et al. (2006) proposed several candidate genes, functionally important for skin pigmentation, in their model for the evolutionary genetic architecture of human pigmentation in the three major human populations. The *MC1R* and *OCA2* genes, showing relatively strong signatures for natural selection, are also included among the candidate genes affecting skin lightening in East Asian populations. Similarly, Myles et al. (2007) used both a long-range haplotype test and an F_{ST}-based approach of pigmentation genes and identified the *DCT* (dopachrome tautomerase, or tyrosine-related protein 2) gene as a candidate gene for recent positive selection in the Chinese. However, no nonsynonymous SNPs have been found within the *DCT* gene to date. Interaction between the *MC1R* and *OCA2* genes may be important in skin pigmentation. Akey et al. (2001) genotyped three SNPs in the *MC1R* gene and two SNPs in the *OCA2* gene in a Tibetan population and identified a significant interactive effect of V92M in the *MC1R* gene and IVS13–15 in the *OCA2* gene on skin pigmentation. These findings suggest that polymorphisms in introns may also be important for skin pigmentation. According to the HapMap datasets, there are some nearly Asian-specific mutations (such as rs12438490, rs12440330, rs12442916, rs1545397, rs12439410, rs12440423, etc.) in intronic regions of the *OCA2* gene. These mutations are observed at extremely high frequencies only in Chinese and Japanese. Further comprehensive analysis including the study of polymorphisms in introns will be needed to understand skin pigmentation in Asians.

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