J Hum Genet (2007) 52:690–693
DOI 10.1007/s10038-007-0167-9

SHORT COMMUNICATION

*OCA2*481Thr*, a hypofunctional allele in pigmentation, is characteristic of northeastern Asian populations

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Received: 11 April 2007 / Accepted: 24 May 2007 / Published online: 14 June 2007

Abstract Asians as well as Europeans have light skin, for which no genes to date are known to be responsible. A mutation, Ala481Thr (c.G1559A), in the oculocutaneous albinism type II (*OCA2*) gene has approximately 70% function of the wild type allele in melanogenesis. In this study, the distribution of the mutation was investigated in a total of 2,615 individuals in 20 populations from various areas. *OCA2*481Thr* prevailed almost exclusively in a northeastern part of Asia. The allele frequency was highest in Buryat (0.24) in Mongolia and showed a north–south downward geographical gradient. These findings suggest that *OCA2*481Thr* arose in a region of low ultraviolet radiation and thereafter spread to neighboring populations.

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

Introduction

Skin color is a complex genetic trait that has long intrigued biologists. Its variation among indigenous peoples is obvious and correlated with levels of ultraviolet radiation, becoming lighter in more northerly latitudes (Jablonski and Chaplin 2000). Asians as well as Europeans have light skin, for which no genes to date are known to be responsible. Recent interest in signatures of positive selection in candidate pigmentation genes has been raised by a long-range haplotype test and an F_{ST}-based approach (Izagirre et al. 2006; Lao et al. 2007; Myles et al. 2007). Some candidate genes appear to have evolved independently after the divergence of Europeans and East Asians. The *OCA2* gene, showing relatively strong signatures for natural selection, is included among the candidate genes affecting

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skin lightening in East Asian populations (McEvoy et al. 2006; Norton et al. 2007).

The human *OCA2* gene (GenBank GeneID: 4948; accession numbers: NM_000275 and NT_000015), the homologue 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 (OCA2). The *OCA2* gene spans 350 kb on chromosome 15q11.2–q12 and consists of 24 exons, encoding an 838-amino acid polypeptide (Lee et al. 1995; Oetting et al. 2005; Puri et al. 2000). A c.G1559A (Ala481Thr; unregistered in Entrez SNP) mutation in exon 14 was first discovered as a compound heterozygote in a European–American patient with apparent autosomal recessive ocular albinism, and its frequency was estimated to be 0.01 in the testing of 50 unrelated normal Caucasian subjects (Lee et al. 1994). Thereafter, this mutant allele, *OCA2*481Thr*, has sporadically been observed in Japanese OCA2 patients (Saitoh et al. 2000; Kato et al. 2003; Suzuki et al. 2003a; Kawai et al. 2005; Ito et al. 2006) and a German patient with congenital cataract and macular hypoplasia (Graw et al. 2006). A transfection study showed that *OCA2*481Thr* had approximately 70% function of the wild type allele in melanogenesis and confirmed it was a relatively mild OCA2 allele (Sviderskaya et al. 1997). This allele is not rare, but has been observed at a frequency as high as 0.10 in Japanese OCA patients and 0.12 in normally pigmented Japanese volunteers (Suzuki et al. 2003b), and it is warned that a number of subclinical patients of OCA2 with this allele might exist not only in Japan, but also all over the world (Kawai et al. 2005). However, as there have been only two studies on distribution of the A481T mutation, it remains unknown whether *OCA2*481Thr* exists in other populations. In this study, therefore, we investigated the frequency of *OCA2*481Thr* in more than 2,600 people from 20 African and Eurasian populations.

Subjects and methods

DNA samples were obtained from 2,615 unrelated individuals living in various areas of Eurasia (Table 1; Fig. 1). The donors were selected at random, irrespective of skin color, and their phenotypic data were unavailable. This study was approved by the Ethical Committee at the Faculty of Medicine, Tottori University. The typing of the A481T mutation was carried out based on the principle of the amplified product length polymorphism (APLP) method (Watanabe et al. 1997). The sequences of the primers used are as follows: OCA2-481-Thr: 5′-atatCAC AAACATTGGAGGtGCTA-3′ (nucleotide positions 11 5895-115914; lower cases are non-complementary nucleotides), OCA2-481-Ala: 5′-CACAAACATTGGAGGA aCTG-3′ (115895-115914) and OCA2-481-R: 5′-TTGGAA ACAATAATGACATTTGG-3′ (115957-115935). The PCR cocktail consisted of 100 µl of Multiple PCR Master Mix from a Multiplex PCR Kit (Qiagen), 20 µl of three primers (1:3:1) with a concentration of 10 pmol/µl, and 80 µl of water. 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 10 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 30 s, and a final extension step of 10 min at 72°C. The products were separated using a polyacrylamide gel (9%T, 5%C), then visualized by ethidium bromide staining. The homozygotes of *OCA2*481Thr* and *OCA2*481Ala* showed 67 and 63-bp bands, respectively, whereas the heterozygotes had both bands. Allele frequencies were estimated and Hardy–Weinberg equilibrium was tested using Arlequin program version 3.01 (Excoffier et al. 2005).

Results and discussion

Table 1 summarizes differences in the distribution of the A481T genotypes and allele frequency for the mutant in 20 populations. In each population, there was no statistically significant departure from Hardy–Weinberg equilibrium (data not shown). The highest value of the *OCA2*481Thr* frequency was observed in Buryat (0.24) and the second highest in Khalha (0.13). The frequencies in northern Han Chinese in Shenyang, Koreans, and Japanese were similar to each other and ranged from 0.075 in Tottori to 0.057 in Okinawa. These values are lower than the data (0.12) in Nagoya, central Japan (Suzuki et al. 2003). A slight but significant difference in distribution was observed between Okinawa and Nagoya ($\chi^2 = 3.92$, $P < 0.05$). In contrast, other Han Chinese populations were either low or zero. This allele was not found in Indo-European and African populations except for Turks in Germany. A recent study also failed to detect *OCA2*481Thr* in about 3,000 European descendants in Australia (Duffy et al. 2007). The frequency (0.01) in Caucasians reported previously (Lee et al. 1994) must have been overestimated. Thus, *OCA2*481Thr* is characteristic of northeastern Asian populations.

Buryat in Mongolia showed the highest frequency, but there may be some populations with higher frequencies in northeast China, Siberia, and/or Central Asia. Anyway, *OCA2*481Thr* is nearly restricted to a northeastern part of Asia. The distribution of *OCA2*481Thr* reflects the migration of ancient northeastern Asians. This allele must have occurred recently in a region of low ultraviolet radiation and have spread to northeast and Far East Asia. It

Table 1 Distribution of A481T Genotypes and *OCA2*481Thr* frequencies

No.	Population	Collection place	*n*	Genotypes of A481T			Frequency of *OCA2*481Thr*
				A	A/T	T	
1	Buryat	Dashbalbar	143	79	59	5	0.241
2	Khalha	Ulaan Baator	173	131	39	3	0.130
3	Korean	Seoul	139	120	19	0	0.068
4	Korean	Kwangju	141	121	19	1	0.074
5	Japanese	Yamagata	289	251	37	1	0.067
6	Japanese	Tottori	179	152	27	0	0.075
7	Japanese	Okinawa	87	78	8	1	0.057
8	Han Chinese	Shenyang	103	89	14	0	0.068
9	Han Chinese	Beijin	40	37	3	0	0.038
10	Han Chinese	Xi'an	109	101	8	0	0.037
11	Han Chinese	Wuxi	119	117	2	0	0.008
12	Han Chinese	Changsha	101	96	5	0	0.025
13	Han Chinese	Putien	118	112	6	0	0.025
14	Han Chinese	Huizhou	111	111	0	0	0.000
15	Indian	New Delhi	107	107	0	0	0.000
16	Turk	West Germany	200	195	5	0	0.013
17	German	West Germany	291	291	0	0	0.000
18	French	Rheims	98	98	0	0	0.000
19	Nigerian	West Germany	31	31	0	0	0.000
20	Ghanaian	West Germany	36	36	0	0	0.000

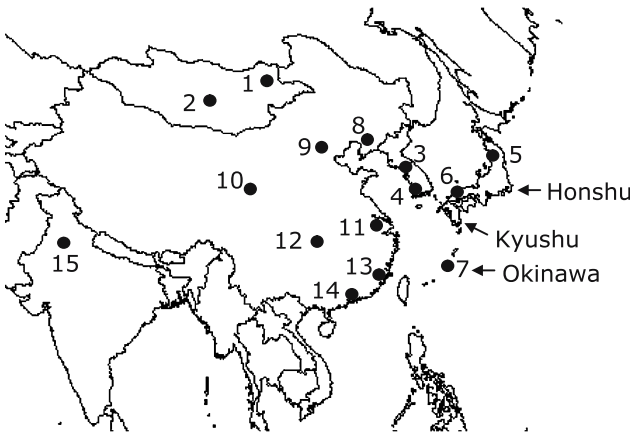

Fig. 1 Location of Asian populations analyzed in the present study

showed a north–south downward geographical gradient. There is a significant correlation between allele frequencies and degrees of latitude in 14 East Asian populations ($r = 0.776$, $P < 0.01$). However, the frequencies are slightly higher in Korean and Japanese than in Han Chinese. This difference in distribution reminds us of the Y-chromosomal haplogroup C, frequencies of which differ remarkably between Han Chinese and northeast Asians including Buryat and Mongolian populations. Koreans and Japanese have higher frequencies than Han Chinese. *OCA2*481Thr* has also made less contribution to the gene pools of Chinese Han populations than those of Far East populations, as haplogroup C could not expand extensively into mainland China due to the rapid and striking northward migration and expansion of haplogroup O from south China (Hammer et al. 2006; Xue et al. 2006). *OCA2*481Thr* has extended from northeast Asia through the Korean peninsula to the Japanese archipelago. The somewhat low frequency in Okinawa suggests a relatively weak gene flow from Kyushu to Okinawa. An east–west decreasing cline may also be observed in Eurasia. It is necessary to investigate the distribution in more detail and whether *OCA2*481Thr* in Europeans has the same origin as that in Asians. These expansions may also be a result of a certain selective advantage of hypofunction in pigmentation.

Individuals homozygous for *OCA2*481Thr* appear phenotypically normal (Suzuki et al. 2003b; Ito et al. 2006). However, this allele may affect variation in skin color in Japanese because of its hypofunction in pigmentation. Japanese skin type is classified on the basis of an individual's susceptibility to sunburn and ability to tan: type I, always burns, never tans; type II, moderately burns, moderately tans; type III, never burns, always tans (Satoh and Kawada 1986). Subjects with type I showed statistically higher prevalence rates for actinic keratosis and nonmelanoma skin cancer in comparison with subjects

having the other types. The incidence of type I was estimated to be 18.6% in Kasai City, western Japan and 10.3% in Ie Island, Okinawa (Naruse et al. 1997; Nagano et al. 1999). These values coincide with total values of the homozygote and heterozygote for *OCA2*481Thr* in Honshu and Okinawa. Individuals with *OCA2*481Thr* may have less resistance to the stress of sunburn (Kawai et al. 2005).

Recently, mutant alleles in the melanocortin 1 receptor (*MC1R*), the membrane-associated transporter protein (*SLC45A2*), and a putative cation exchanger protein (*SLC24A5*) have been elucidated to play a key role in light skin and its variation in European populations (review, Sturm 2006). In contrast, little is understood about the genetic and molecular basis of light skin color in Asian populations. *OCA2*481Thr* described here is, of course, not a key mutation responsible for light skin pigmentation in Asians.

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