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ORIGINAL ARTICLE

Population differences of two coding SNPs in pigmentation-related genes *SLC24A5* and *SLC45A2*

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Abstract The two genes *SLC24A5* and *SLC45A2* were recently identified as major determinants of pigmentation in humans and in other vertebrates. The allele p.A111T in the former gene and the allele p.L374F in the latter gene are both nearly fixed in light-skinned Europeans, and can therefore be considered ancestry informative marker (AIMs). AIMs are becoming useful for forensic identification of the phenotype from a DNA profile sampled, for example, from a crime scene. Here, we generate new allelic data for these two genes from samples of Chinese, Uygurs, Ghanaians, South African Xhosa, South African Europeans, and Sri Lankans (Tamils and Sinhalese). Our data confirm the earlier results and furthermore demonstrate that the *SLC45A2* allele is a more specific AIM than the *SLC24A5* allele because the former clearly distinguishes the Sri Lankans from the Europeans.

Keywords Skin color · Pigmentation · Population differentiation · *SLC24A5* · *SLC45A2*

Introduction

The diversity of human physical characters has long intrigued mankind. Among such characters, the variations in pigmentation of the hair, eyes, and skin are the most obvious phenotypes that distinguish human populations. The variation of skin pigmentation is logical when UV radiation is considered because dark skin offers more protection from UV irradiation at or near the equator, and pale skin protects people from vitamin D deficiency at higher latitudes.

The genes that are known to affect melanogenesis and human pigmentation diseases have been identified through the study of pigmentation mutants of various species. Among them, *AIM-1* (*SLC45A2*, *MATP*, BC064405) was identified to be responsible for several pigmentation mutations such as the orange-red variant (*b*) of medaka fish and the underwhite gene (uw) of mice [7, 15]. The human homologue of this gene was identified as the causal gene of oculocutaneous albinism 4 (MIM# 606202). We investigated the allele frequencies of two nonsynonymous polymorphisms, c.814G>A and c.1122G>C, resulting in p.E272K (refSNP ID: rs 26722) and p.L374F (refSNP ID: rs16891982), respectively, in several human populations. The derived 374F allele is almost fixed in people of European descent, but is absent or quite rare in the other populations examined [14, 24]. In addition, these single nucleotide polymorphisms (SNPs) in the gene were reported to be associated with normal human pigmentation variations, i.e., 374L is significantly associated with dark hair, skin, and eye color in Europeans [8]. Recently, we analyzed the sequence variations of *SLC45A2* and found that a selective sweep (genetic variation around the site of directional selection is reduced because linked alleles undergo "hitchhiking" and also become fixed) in the region encompassing p.L374F was observed only in Europeans [22].

More recently, positional cloning, knockdown, rescue, and expression analysis have shown the SLC24A5 (NM_205850) to be responsible for a zebra fish pigmentation variant, the golden phenotype [12]. It is interesting that a coding SNP, p.A111T (refSNP ID: rs1426654), of the human orthologue of this putative cation exchanger gene also shows a population-specific pattern similar to p.L374F

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of *SLC45A2*, i.e., the derived 111T allele is nearly fixed in Europeans. In addition, the heterozygosity of the genomic region around *SL24A5* was decreased in Europeans.

An ancestry informative marker (AIM) is a human polymorphism that exhibits substantially different frequencies between populations, and *SLC24A5* is a good example of AIM as reported previously [19]. To understand the evolution and evaluation as AIMs of these two pigmentation-related genes, the allelic frequency of both p.L374F of *SLC45A2* and p.A111T of *SL24A5* in several human populations over the world was investigated.

Materials and methods

DNA samples

The study protocol was approved by the ethical committee of Kurume University. Randomly selected individuals from populations sampled were Chinese from Guangzhou (South China), Uygurs from Urumqi (West China), Ghanaians from Accra, Xhosans (Africans) and European-Africans from Cape Town, and Sinhalese and Tamils from Sri Lanka. The numbers of samples analyzed were 80 for Chinese, 121 for Ghanaians, 101 for Xhosans, 101 for Europeans, 55 for Uygurs, 54 for Sinhalese, and 58 for Tamils.

Polymerase chain reaction-restriction fragment length polymorphism analysis to identify p.A111T polymorphism

The region containing p.A111T was amplified in 20 μl of 1× buffer containing 1 U of TAKARA *Ex* Taq polymerase (Takara, Tokyo, Japan). The primers used were 5′-GAAG CTGTAGCTTTGAGTATC-3′ and 5′-CACACTGAGTA AGCAAGAAGT-3′ (from International HapMap project homepage, http://www.hapmap.org/cgi-perl/snp_details?name=rs1426654). The temperature profile was denaturation at 96°C for 3 min, followed by 35 cycles of denaturation at 96°C for 15 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s. The resultant PCR products were treated with *Hha*I (Takara) to digest the restriction site in alleles containing 111A; they were then separated by electrophoresis.

Denaturing high-performance liquid chromatography (DHPLC) was performed to identify the p.L374F polymorphism of *SLC45A2* as described previously [22].

Results and discussion

The allele frequency of p.A111T of the *SLC24A5* gene was determined in Chinese; Sinhalese and Tamils from Sri Lanka; Uygurs, Europeans, and Xhosans (Africans) from South Africa; and Ghanaians with the use of polymerase chain reaction-restriction fragment length polymorphism. As shown in Fig. 1, the frequency of p.A111T in European and African populations was equivalent to that of reported data [12, 19], i.e., 111T was overrepresented only in the European population (0.975). In addition, samples of the three Asian populations, Chinese of east Asia, Sri Lankans of south Asia, and Uygurs of central Asia were examined. The frequency for the Chinese (0.019 for 111T) was similar to previous studies [12, 19]. On the other hand, the frequency of 111T allele was relatively high in both the Sri Lankan and Uygur populations: 0.500, 0.293, and 0.536 for Sinhalese, Tamils, and Uygurs, respectively.

We also determined the frequency of p.L374F of the *SLC45A2* gene in Uygurs by DHPLC analysis in this study and the 374F allele were found in relatively high frequency in Uygrus. The *SLC45A2* results, except for those of Uygurs, are in agreement with results from a previous study [22].

A previous observation of gene frequency data for 18 protein and blood group loci suggested that Sinhalese are

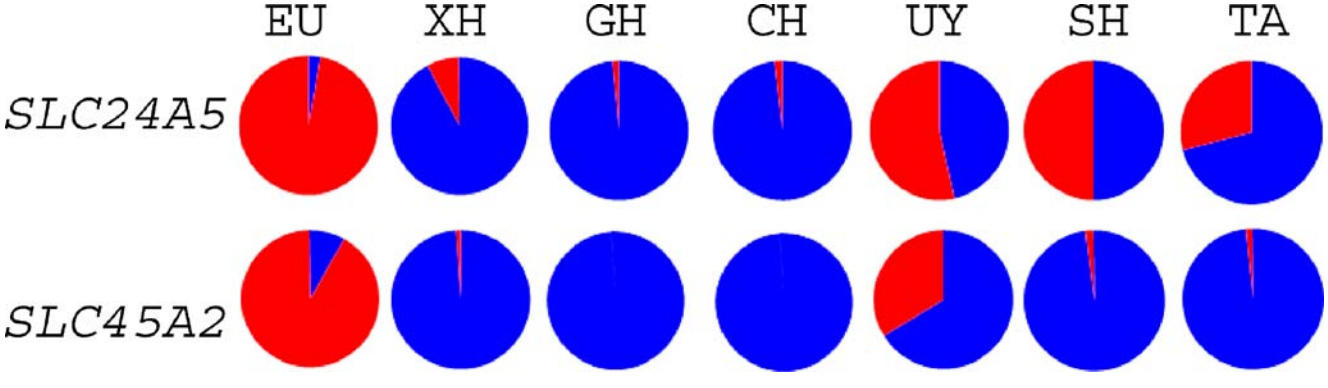

Fig. 1 SNP allele frequencies in *SLC24A5* and *SLC45A2*. Each row of seven *circles* represents the distribution of alleles in Europeans (*EU*) and Xhosans (*XH*) in South Africa, Ghanaians (*GH*), Chinese (*CH*), Uygurs (*UY*), and Sinhalese (*SH*) and Tamils (*TA*) in Sri Lanka. Ancestral alleles (111A of *SLC24A5* and 374L of *SLC45A2*) are indicated by *blue*, and derived alleles (111T of *SLC24A5* and 374F of *SLC45A2*) are indicated by *red*

closer to Iranians and Afghans than to Mongoloids [18] and that the constitutions of the *FUT2* allele of Sinhalese and Tamils are similar [21]. Thus, there is little or no genetic flow from neighboring East or Southeast Asian populations.

The distributions of several genetic markers used in these studies suggested that Uygurs have a considerable admixture of European and East Asian genes [5, 10, 13]. We also studied variations of the *FUT2* gene and found that they suggested an admixture in the same Uygur samples [17]. The intermediate frequency distribution of European type alleles, 111T of *SLC24A5* and 374F of *SLC45A2*, confirm the admixture of European and East Asian genes in the Uygur population.

Short tandem repeat analysis and fingerprinting are powerful forensic tools for identification of individuals using residual samples of blood, hair, etc. and for paternity tests [6, 9, 23]. However, such methods are only useful for the purposes of identification when the suspect has been identified or when the genotype or pattern matches one already present on the DNA database. In addition to these markers, SNPs are now useful in the forensic field because the simplicity and population specificity of SNPs allow inferences to be drawn about the population origin of interest for forensic purposes [3, 4, 11, 20, 16]. Recently, research targeted toward identifying specific genetic markers that may provide further information on the physical characteristics of the individuals from which the sample came [2] has identified markers such as AIMs. AIMs are becoming useful for forensic identification of the phenotype from a DNA profile sampled, for example, from a crime scene. Both *SLC24A5* and *SLC45A2* are good examples of AIMs as reported for *SLC24A5* previously [19]. When compared to the results of the *SLC45A2* gene, the values were in accordance with that of *SLC24A5* in all populations studied except Sri Lankan; both Sri Lankan populations showed different patterns in frequency between the two genes (Fig. 1). These data demonstrate that the *SLC45A2* allele is more specific AIM than the *SLC24A5* allele, because the former clearly distinguishes the Sri Lankans from the Europeans.

The products encoded by both the *SLC24A5* and *SLC45A2* genes are predicted to function as transporters and may take part in melanin synthesis [7, 12]. In addition to the fish mutants of both genes, there is evidence of correlation between the genotypes and skin pigmentation in humans [8, 12], although only the *SLC45A2* gene has been associated with albinism at present. We hypothesize that directional selection has acted or is acting on *SLC45A2* in the European population because of evidence for a selective sweep in the region surrounding p.L374F. It is also possible that similar natural selection has acted or is acting on the *SLC24A5* gene in the European population for the same reason [12].

Skin reflectance is lowest at the equator, then gradually increases [1]. According to the traditional skin color map by Biasutti, Sri Lanka is within 20° of the equator and categorized as darker skin color [1] (http://anthro.palomar.edu/adapt/adapt_4.htm). There are several scenarios to explain the differences in allele frequency of the coding SNPs between the *SLC24A5* and *SLC45A2* genes in South Asian populations. One of them is that 374L has a selective advantage against harmful ultraviolet radiation (UVB), and the frequency of the 374F allele migrating from Central Asian populations might have been reduced by purifying selection in South Asian populations. This may mean that the functional constraint is stronger on *SLC45A2* than on *SLC24A5*. Sequence analysis of the *SLC24A5* gene flanking the p.A111T polymorphism and functional analysis of the coding SNPs will help us understand the evolution of these two pigmentation genes.

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