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SHORT COMMUNICATION

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Distinctive distribution of *AIM1* polymorphism among major human populations with different skin color

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Abstract The genetic background for human skin color has been a major topic in human genetics; however, its molecular basis is still unclear. The gene for the AIM-1 protein (*AIM1*) was recently found to be responsible for the body color of medaka fish. In the search for the genes controlling human skin color variations, we have investigated genetic polymorphisms of this gene, and we have found a single-nucleotide polymorphism that has clear association with major human populations in terms of skin color.

Key words *AIM1* · Melanin synthesis · Population study · Primates · Skin color diversity · SNPs

Introduction

Among many genes affecting human melanin synthesis, only the melanocortin 1 receptor gene (*MC1R*) can explain nonpathogenic intrapopulation skin color variations; however, interpopulation diversity was not addressed (Flanagan et al. 2000; Harding et al. 2000). Human AIM-1 was first identified as a melanoma antigen with unknown function (Harada et al. 2001). However, recently, Newton et al. (2001) assigned it to the *underwhite* locus (*uw*), which underlies a new type of oculocutaneous albinism. *AIM1* is a newly identified gene in fish that controls melanin synthesis; *B* (homologue of the human *AIM1*) mutants of medaka fish displayed hypopigmentation with different phenotypes (Fukamachi et al. 2001). The function of AIM-1 as a transporter in melanin synthesis has been suggested by its 12-domain membrane-pass structure, which is similar to the structure of the sucrose transporter in plants (Fukamachi et al. 2001).

We have investigated polymorphisms of human *AIM1* among representatives of major human populations with varying degrees of skin pigmentation including white South Africans, Ghanaians, Japanese, and New Guinea Islanders to identify any possible relation to skin color variation.

Subjects and methods

A total of 205 DNA samples from individuals belonging to major human populations, white South Africans (n = 54), Ghanaians (n = 50), Japanese (n = 49), and New Guinea Islanders (n = 52), were used in this study. All the anonymously coded samples were collected from unrelated individuals with informed consent. For the comparative study, DNAs from a chimpanzee (*Pan troglodytes*), a bonobo (*Pan paniscus*), a gorilla (*Gorilla gorilla*), an orangutan (*Pongo pygmaeus*) and a Japanese macaque (*Macaca fuscata*) were also used.

For the identification of molecular alterations in the *AIM1* coding region, primers were designed to amplify seven exons of *AIM1* separately (Table 1). After polymerase chain reaction (PCR) amplification, we then determined the nucleotide sequences of each DNA fragment of the PCR product by using an ABI PRISM BigDye Terminator cycle sequencing FS kit and ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Tokyo, Japan).

The genotyping of E272K was performed with a PCR-*Taq*I restriction fragment length polymorphism (RFLP) analysis of exon 3. By using allele-specific forward primers (L374 and F374) and a universal reverse primer (Universal

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R) (Table 1), allele-specific PCR was performed to identify L374F genotypes.

Results and discussion

In our initial study, we have identified two nonsynonymous single-nucleotide polymorphisms in human *AIM1* by determining nucleotide sequences of the coding regions of the gene. They are GAG (glutamate)/AAG (lysine) in codon 272 (E272K) and TTG (leucine)/TTC (phenylalanine) in codon 374 (L374F). The distribution of these two substitutions was screened for in the major human populations by using PCR-*Taq*I RFLP and allele-specific PCR methods. The former substitution, which was found in the database (NCBI dbSNP; rs26722), was polymorphic only in the Japanese and New Guinea Islander populations, and monomorphic (allele frequency: <0.01) in the white South African and Ghanaian populations. The other single nucleotide substitution causing L374F, which showed a distinctive population distribution, existed exclusively in white South Africans but not in the other populations (Table 2). Since white South Africans are admixtures of several European ethnic groups (Thompson 1990), this substitution is regarded as a Caucasian-type allele. The genetic contribution of non-Caucasians to the present white South African population (Caucasians) is estimated to be about 7% from studies of the HLA human major histocompatibility complex (Botha et al. 1975), and this value well accounts for the breakdown of the monomorphic status of Phe (0.89) among the white South Africans in the present study. Newton et al. (2001) also reported that L374F is a common polymorphism.

To identify the prototype of the human allele, we analyzed *AIM1* in several primate species (Figure 1), and the TTG (Leucine) allele was deduced to be the prototype. On the basis of partial nucleotide sequences of *AIM1* in humans, primates, mouse, and medaka, as well as in the celery sucrose transporter (Lemoine 2000), used as an orthologue, predicted amino acid sequences were aligned and compared (Figure 1). This alignment revealed a highly conserved amino acid sequence throughout all species, from primates to plants, and, especially, the ubiquitous presence of leucine in position 374 of AIM-1. The presence of the leucine residue may be critical for the function of AIM-1.

It is of course premature to presume that the hypopigmentation in the Caucasian population is caused by the loss of leucine in amino acid position 374 of AIM-1. However, not a little genetic evidence suggests an intervention of AIM-1 in mammalian pigmentation. The cytogenetic location of human *AIM1* (5p13.3, NCBI LocusLink; 51151) does not preclude *AIM1* from being a candidate for an important pigmentation locus, *uw* (Sweet et al. 1998), and it was, in fact, proved that the *AIM1* is responsible for one type of human albinism (Newton et al. 2001). In mice, a double mutation of *uw* and *Mc1r* results in significantly less pigmentation than either mutation alone (Lehman et al. 2000). It is thus speculated that the combination of poly-

Table 1. Primer sequences

Exon 1	F	5′-CCAGTTTGAAACACAGACCC-3′
	R	5′-TCAAACACATGAACATCCTCC-3′
Exon 2	F	5′-GGCAAGAAGTTTAGGTGGAA-3′
	R	5′-GCTGACCCGTTCATTCA-3′
Exon 3	F	5′-CTGAAGGGGAGTGTCTATGC-3′
	R	5′-CCCCATGAAACTCTTCTCGT-3′
Exon 4	F	5′-CTTTGTGTGATGGCTGACTG-3′
	R	5′-GAGGATAGCCCAGAAGAACC-3′
Exon 5	F	5′-GAGGTGGAGAAGCAGAG-3′
	R	5′-CTGGTATTTTAAACAGTAGGAA-3′
Exon 6	F	5′-TCTTCAGAAGAAACGGATTG-3′
	R	5′-CCAGCCTTCAGATGAGTC-3′
Exon 7	F	5′-TTTGCTGACCTGTGCCCTAA-3′
	R	5′-GCAGATTCCACGGCTGAAAT-3′
L374		5′-TTGGATGTTGGGGCTTG-3′
F374		5′-TTGGATGTTGGGGCTTC-3′
Universal	R	5′-TCCCTTTCATTTTCCAGAGA-3′

Table 2. Allele frequency of L347F

Population	Number of samples	Frequency	
		Leu	Phe
White South African	54	0.11	0.89
Ghanaian	50	1.00	0.00
Japanese	49	1.00	0.00
New Guinea Islanders	52	1.00	0.00

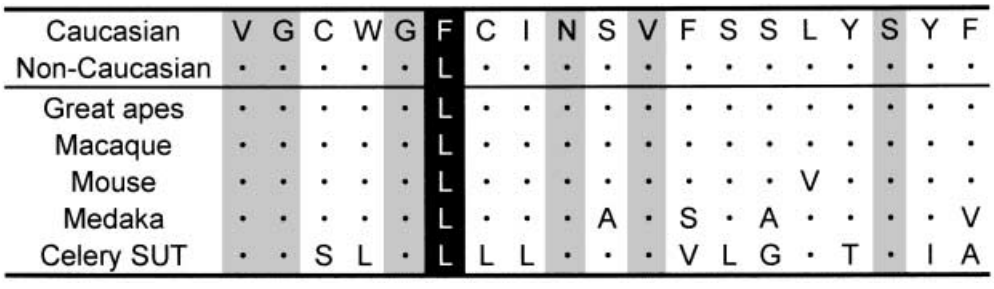

Fig. 1. Amino acid alignment of the L347F site and its flanking regions in AIM-1. Great apes used were a chimpanzee, a bonobo, a gorilla, and an orangutan. Mouse and medaka sequences are from the GenBank database, accession numbers AF360357 and AF332510, respectively. *Celery SUT*, *Apium graveolens* sucrose transporter (AgSUT1, GenBank accession number AF063400). *Black shading* shows the L374F position. In other positions, *dots* indicate residues identical to the human sequence. Gray shadings show conserved residues

morphic alleles at two loci, *AIM1* and *MC1R*, may predict a large part of human skin color diversity.

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References

Botha MC, Campbell E, Briggs B, du Toit ED (1975) Differences on serological definition and frequency of some HLA antigens among southern African populations. In: Kissmeyer-Nielsen F (ed) Histocompatibility testing. Munksgaard, Copenhagen, pp 151–172

Flanagan N, Healy E, Ray A, Philips S, Todd C, Jackson IJ, Brich-Machin MA, Rees JL (2000) Pleiotropic effects of the melanocortin 1 receptor (*MC1R*) gene on human pigmentation. Hum Mol Genet 9:2531–2537

Fukamachi S, Shimada A, Shima A (2001) Mutations in the gene encoding B, a novel transporter protein, reduce melanin content in medaka. Nat Genet 28:381–385

Harada M, Li YF, El-Gamil M, Rosenberg SA, Robbins PF (2001) Use of an in vitro immunoselected tumor line to identify shared melanoma antigens recognized by HLA-A *0201-restricted T cells. Cancer Res 61:1089–1094

Harding RM, Healy E, Ray AJ, Ellis NS, Flanagan N, Todd C, Dixon C, Sajantila A, Jackson IJ, Birch-Machin MA, Rees JL (2000) Evidence for variable selective pressure at MC1R. Am J Hum Genet 66:1351–1361

Lehman AL, Silvers WK, Puri N, Wakamatsu K, Ito S, Brilliant MH (2000) The underwhite (*uw*) locus acts autonomously and reduces the production of melanin. J Invest Dermatol 115:601–606

Lemoine R (2000) Sucrose transporters in plants: update on function and structure. Biochim Biophys Acta 1465:246–262

Newton JM, Cohen-Barak O, Hagiwara N, Gardner JM, Davission MT, King RA, Brilliant MH (2001) Mutations in the human orthologue of the mouse *underwhite* gene (*uw*) underlie a new form of oculocutaneous albinism, OCA4. Am J Hum Genet 69:981–988

Sweet HO, Brilliant MH, Cook SA, Johnson KR, Davisson MT (1998) A new allelic series for the *underwhite* gene on mouse chromosome 15. J Hered 89:546–551

Thompson L (1990) A history of South Africa. Yale University Press, New Haven, CT, USA, pp 31–109