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SHORT COMMUNICATION

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MATP polymorphisms in Germans and Japanese: the L374F mutation as a population marker for Caucasoids

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Abstract Inference of the population and ancestry to which an individual belongs is important in forensic individualization and personal identification. In this study, five polymorphisms of the membrane-associated transporter protein (MATP) gene were investigated in German and Japanese populations. The L374F mutation was present at an allele frequency as high as 0.96 in the German population, whereas it was completely absent in the Japanese population. This extreme difference in allele frequency suggests that the L374F mutation is valuable as a population and ancestry informative marker for Caucasoids.

Keywords Ancestry marker · L374F · Membrane-associated transporter protein · Polymorphism · Population marker

Introduction

Amplification by polymerase chain reaction (PCR) followed by typing of short tandem repeat (STR) polymorphisms and sex-associated sequences has drastically contributed to forensic individualization and personal identification. However, these DNA profiles can provide only limited information on an individual's physical characteristics. Recently, studies on the appearance of individuals have been attempted (Hopkinson et al. 2000; Grimes et al. 2001; Frudakis et al. 2003). Inference of the population and ancestry to which an individual belongs is also important, and good informative markers are needed in the forensic field (Lowe et al. 2001; Forster et al. 2002; Shriver et al. 2003). DXYS156 is a unique STR locus and some alleles are useful to infer paternal and maternal geographic origins (Calì et al. 2002).

The human membrane-associated transporter protein (MATP, GenBank Locus ID: 51151; GenBank accession numbers: NM_016180 and NT_006576) gene, also known as the antigen in melanoma-1 (AIM1) gene, spans 40 kb on chromosome 5p and consists of 7 exons, encoding a 530-amino acid polypeptide. The human MATP gene is associated with pigmentation and is responsible for oculocutaneous albinism type 4 (Newton et al. 2001; Inagaki et al. 2004; Rundshagen et al. 2004). The L374F mutation, resulting from a G-to-C transversion in exon 5 of the MATP gene, has been observed in white South Africans at a frequency as high as 0.89, but not in 3 other investigated populations including Ghanaians, Japanese and New Guinea Islanders. The F374 allele is likely to be associated with the skin pigmentation of the major human populations (Nakayama et al. 2002). Although this substitution can be a good marker for Caucasoids, no further studies have been carried out in normal Caucasoid populations. In this study, five mutations in the MATP gene were investigated in German and Japanese populations.

Materials and methods

DNA samples were obtained from 93 Germans from the Munich area and 103 Japanese from Tottori in western Japan. Genomic DNA was extracted by a salting-out

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Table 1 Mutations investigated in this study and the allele frequencies in German and Japanese populations

Region	Nucleotide position	Nucleotide substitution	Amino acid substitution	Allele frequency		
				Allele	German	Japanese
Exon 3	20880	G>A	E272K	A	0.032	0.383
Intron 3	21002	$(ttg)_{4>5}$	-	5	0.005	0.257
Exon 4	30239	G>A	T329T	A	0.005	0.238
Exon 5	33057	G>C	L374F	C	0.962	-
Exon 7	39923	G>C	V507L	C	-	0.005

method (Miller et al. 1988). This study was approved by the ethical committee at the Faculty of Medicine, Tottori University.

The L374F mutation on exon 5 was determined by amplified product length polymorphism (APLP) analysis (Watanabe et al. 1997; Umetsu et al. 2001). The sequences of the primers are as follows: FG: 5'-GAGGTTG-GATGTTGGaGCTTG-3' (nucleotide positions 33037–33057; lower cases are non-complementary nucleotides), FC: 5'-attatGAGGTTGGATGTTGGGaCTTC-3' (33037–33057) and R1: 5'-GAAGACATCCTTAGGAGAGA-GAAAGAC-3' (33122–33096). PCR was performed using HotStarTaq polymerase, as recommended by the supplier (Qiagen, Hilden, Germany) in 12 µl reactions containing 20 ng genomic DNA, and 2.5 pmol of primers FG and R1 and 3.75 pmol of primer FC. Cycle conditions were 95°C for 15 min, then 30 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 40 s, with a final extension step of 10 min at 72°C. The PCR products were separated on native polyacrylamide gels (9%T and 5%C). The homozygotes of the L374 and F374 alleles showed 86 bp and 91 bp bands, respectively, whereas the heterozygotes consisted of both bands. Three additional mutations, E272K, T329T and V507L on exons 3, 4 and 7, were investigated by restriction fragment length polymorphism (RFLP) analysis (Table 1). The sequences of the primers were as follows: E3F: 5'-AGAGGTTGCAAAGGG-CATTC-3' (20795–20814) and E3R: 5'-CCCAT-GAAACTCTTCTCGTCAA-3' (21064–21043) for E272 K, E4F: 5'-GTGAACATGCCTCCTCACTACC-3' (30183–30204) and E4R: 5'-CGTTCATTACCTGGCC-CATGA-3' (30294–30274) for T329T and E7F: 5'-TGCAGCTGGCTCAGATCCT-3' (39852–39870) and E7R: 5'-AGCGACAAAGCAACAGCCTATC-3' (39973–39952) for V507L. PCR of each fragment was performed as described earlier except for 2.5 pmol of each primer and annealing at 55°C. The products for exons 3, 4 and 7 were digested with the restriction enzymes *Taq* I, *Bsu*R I and *Mva* I (Fermentas, Hanover, MD), respectively. Because the fragments for exon 3 contained an STR in the 3'-flanking region, the products were analyzed by 4% denaturing polyacrylamide gel electrophoresis. The nucleotide numbering was according to the genomic sequence (GenBank accession number NT_006576). The adenine of the translation initiation codon ATG and the guanine of the stop codon TAG correspond to nucleotides 62 and 39997, respectively. Estimation of the allele and haplotype frequencies was performed using the EH (Estimating Haplotype-frequencies) program (http://link-age.rockefeller.edu/soft/).

Results and discussion

A total of 5 mutations including 4 single-nucleotide polymorphisms on exons 3, 4, 5 and 7, and 1 STR polymorphism on intron 3 were investigated in this study. Table 1 shows the allele frequencies of each mutation site of the MATP gene. The distribution of genotypes observed was in agreement with Hardy-Weinberg equilibrium. The allele frequencies were significantly different between the German and Japanese populations except for those of exon 7. The L374F mutation was observed at an extremely high frequency in the German population, but not in the Japanese population at all. All Germans examined in this study were either homozygous or heterozygous for the F374 allele. The frequency was as high as 0.962, which is comparable to the frequency (0.957) obtained by a study of 176 unrelated German patients with symptoms of albinism (Rundshagen et al. 2004). These values were rather high in comparison with that of 0.89 found in white South Africans (χ^2=6.10, df=1, P<0.02). This difference may be due to the fact that white South Africans are admixtures of several European ethnic groups and contain 7% non-European genes (Nakayama et al. 2002). In the three other mutation sites, the German population showed low diversity. The increase in the number of repeat units from $(ttg)_4$ to $(ttg)_5$ in intron 3, and the G-to-A transition in T329T in exon 4 were present in 4 out of 176 patients with albinism (allele frequencies 0.011). The G-to-A transition in E272K was also observed in one patient (Rundshagen et

Table 2 MATP haplotypes in German and Japanese populations

Haplotype	Site					Frequency	
	20880	21002	30239	33057	39923	German	Japanese
1	G	4	G	G	G	0.005	0.354
2	G	4	A	G	C	-	0.005
3	G	5	G	G	G	-	0.055
4	G	5	A	G	G	0.005	0.202
5	A	4	G	G	G	0.027	0.353
6	A	4	A	G	G	-	0.031
7	G	4	G	C	G	0.957	-
8	A	4	G	C	G	0.005	-
Haplotype diversity						0.084	0.709

al. 2004). The frequencies were similar to those of our German sample.

Table 2 shows the haplotypes and their frequencies in both populations and 5 of the 8 haplotypes were observed in the German sample. However, the haplotype diversity (*h*) was very low, and the haplotype 7 with a cytosine at nucleotide 33057 dominated. This major haplotype must have occurred after divergence between the Caucasoids and Mongoloids. The haplotype 8 may have resulted from a recombination event between the haplotypes 5 and 7. The haplotypes 1, 4 and 5 without cytosine at the same position may represent the remnants of genes of early populations that moved to Europe and/or the gene flow from Asia to Europe after divergence.

In conclusion, the comparison between Germans and Japanese showed that the F374 allele in the MATP gene was exclusively present in the German sample. This allele could be a valuable marker for the identification of Caucasoids in the forensic field. However, iris, skin and hair color are diverse among Caucasoid populations. The F374 allele may have prevailed as a consequence of natural selection, i.e., adaptation to a smaller amount of solar ultraviolet radiation. Prior to practical application, more data are needed on the geographical distribution of this polymorphism on a worldwide level. The simple, rapid and economical APLP method presented in this study would be very useful to accumulate frequency data on the L374F mutation.

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