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ORIGINAL ARTICLE

Variants of the melanocortin 1 receptor gene (*MC1R*) and *P* gene as indicators of the population origin of an individual

Sosuke Masui · Masato Nakatome · Ryoji Matoba

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Abstract The population origin of an individual is often requested to be determined from specimens left at a crime scene for identifying a suspect and individual identity. The melanocortin 1 receptor gene (*MC1R*) and *P* gene are associated with human pigmentation. Although several studies have reported that these genes are highly polymorphic in human populations, it is unclear if the allele variants can be used to determine the population origin of an individual. We aimed to determine the ethnic origin of an individual by using single nucleotide polymorphisms (SNPs). Eighteen SNPs in the *MC1R* gene and *P* genes were genotyped in 52 individuals by the direct sequencing method, and 4 SNPs (*MC1R* gene: R163Q and *P* gene: IVS5+1001, IVS13+113, and H615R) were selected on the basis of differences in frequencies. Subsequently, we genotyped these four SNPs in 422 volunteers from six ethnically defined populations using a polymerase chain reaction-based assay. The results revealed that the allele variants were present with high frequencies in Asian populations but were low in European and African populations. On the basis of these results, we defined a specific combination of a genotype (R163Q) and a diplotype group (IVS5+1001, IVS13+113, and H615R). This study indicates that the specific combination of a genotype and a diplotype group would be effective in estimating the population origin of an individual from a list of population groups.

Keywords *MC1R* gene · *P* gene · SNPs · The population origin of an individual

Introduction

Forensic medicine often requires that the population origin of a particular individual is estimated from specimens such as blood, saliva, semen stains, bones, and tissues left at a crime scene. The population origin of an individual could be valuable information to identify a suspect. Therefore, genetic loci with large differences in frequency among ethnically defined populations, such as haplogroups of mitochondrial DNA (mtDNA) [1–3], microsatellites [4–7], and single nucleotide polymorphisms (SNPs) [8, 9], have been studied. However, to date, the genetic markers that determine the population origin of an individual have not been confirmed.

Here, we focused attention on human hair, skin, and iris pigmentation. These are highly visible traits that differ among ethnic groups and could be useful for determining the ethnic origin of an individual. These traits are due to varied amounts of eumelanin (brown and black melanin) and phaeomelanin (red and yellow melanin) produced by melanocytes. Consequently, we realized that investigation of the allele variants of the human melanocortin 1 receptor gene (*MC1R*) and human *P* gene that are associated with melanogenesis and confirmation of SNPs in these genes could be useful for estimating the population origin of an individual.

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S. Masui (✉)
Forensic Science Laboratories, Shiga Police Headquarters,
1-34-3 Karasaki,
Otsu, Shiga 520–0106, Japan
e-mail: momotaro1228@gaia.eonet.ne.jp

M. Nakatome · R. Matoba
Department of Legal Medicine, Osaka University Graduate School of Medicine Division of Preventive and Environmental Medicine, 2–2 Yamadaoka,
Suita, Osaka 565–0871, Japan

The *MC1R* gene, which is located at chromosome 16q24.3, encodes a 7-pass transmembrane G-protein-coupled receptor that is 317 amino acids in length. This protein has a high binding affinity for the alpha-melanocyte-stimulating hormone (α-MSH) and regulates eumelanin and phaeomelanin production in melanocytes [10–12]. Several variants of the *MC1R* gene are strongly associated with the red hair and fair skin phenotype observed in Caucasian groups [13–18]. Moreover, there have been many reports regarding the relationship between *MC1R* variants and an increased risk of skin cancer [19–21]. On the other hand, the *P* gene, which is located at chromosome 15q11.2–q12, is a homologue of the mouse pink-eyed dilution gene and encodes an 838-amino acid protein (P protein) containing 12 apparent transmembrane domains [22–25]. The P protein is integral to the melanosomal membrane, although the biological function of this protein is unknown. In addition, to date, several potential functions, such as a membrane transporter of a substrate [26] and a sorter of tyrosinase to the melanosome [27], have been proposed for the P protein. Mutations in the *P* gene are associated with oculocutaneous albinism type II (OCA2), which is the most common type of human albinism [28–31], and phenotypic variations in human iris color [32, 33].

In this study, we preliminarily genotyped 18 SNPs in the *MC1R* and *P* genes by the direct sequencing method and selected four SNPs (*MC1R* gene: R163Q [SNP1] and *P* gene: IVS5 + 1001, IVS13 + 113, and H615R [SNP2, SNP3, and SNP4, respectively]) on the basis of differences in frequencies. Subsequently, we tested the hypothesis that the specific combination of a genotype (SNP1) and a diplotype group (SNP2–SNP4) might be effective for estimating the population origin of an individual.

Materials and methods

Isolation of genomic DNA

The genomic DNA of 422 unrelated individuals (200 Japanese [JPN], four Korean [KRE], 24 Chinese [CHN], 65 Mongolian [MGL], 77 European Caucasians [EUR], and 52 African Negroes [AFR]) was isolated from buccal cells or whole blood using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany). The Osaka University Ethics Committee approved the study protocol. Informed consent was obtained from all the volunteers enrolled in this study.

SNP genotyping

Sequencing analysis

As a preliminary study, 18 SNPs (11 SNPs in the *MC1R* gene and seven SNPs in the *P* gene) were chosen from the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/index.html) (Table 1). The genotypes of these SNPs were confirmed in 52 individuals (20 JPN, four CHN, 25 EUR, and three AFR) by the direct sequencing method using an ABI 310 Genetic Analyzer and the BigDye™ Terminator Cycle Sequencing Kit ver.3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Polymerase chain reaction-amplified product length polymorphism analysis

The four SNPs selected by the preliminary sequencing study (See Supplementary Table S1) were genotyped in the 422 volunteers by the polymerase chain reaction (PCR)-amplified product length polymorphism (APLP) method [3, 34], which was much easier and simpler than the sequencing method. This method is a PCR-based assay in which nucleotide substitutions are detected as amplified fragments of different lengths. The PCR-APLP primers were prepared on the basis of the nucleotide sequences of the human *MC1R* and *P* genes and designed with an allele-specific nucleotide at the 3′ end and several noncomplementary nucleotides (poly-Ts) at the 5′ end (See Supplementary Table S1). The PCR product sizes were controlled by varying the number of poly-Ts added to the 5′ end of the primers.

PCR was performed using a reaction mixture of 50 μl containing 10 ng genomic DNA, 20 mM ammonium sulfate, 4.0–16.0 pmol PCR-APLP primers, and 25 μl One Shot LA PCR™ Mix (Takara Bio, Otsu, Japan). The products were amplified using a DNA thermal cycler (GeneAmp PCR System 9700, Applied Biosystems), and the PCR conditions were as follows: 96 °C for 2 min; 32 cycles of 96°C for 1 min, 57–63°C for 1.5 min, 70°C for 2 min; and a final extension at 72°C for 10 min. The PCR products were separated by electrophoresis for 40 min at 100 V in a 2.0% agarose gel and directly visualized with ethidium bromide under UV illumination.

Statistical analysis

Hardy–Weinberg equilibrium

Significant deviations from the Hardy–Weinberg equilibrium (HWE) were revealed for the four selected SNPs (See Supplementary Table S1) by a computation performed using the software package for population genetics ARLEQUIN ver.3.1 (http://cmpg.unibe.ch/software/arlequin3) according to the procedure of Guo et al. [35]. The computation was performed using a method analogous to Fisher's exact test and a modified version of the Markov-chain random walk algorithm.

Table 1 Eighteen SNPs in the *MC1R* and *P* genes and the allele frequencies confirmed by preliminary sequencing analysis

Gene	SNP description	dbSNP accession no.	Allele	Allele frequency[a]			
				JPN	CHN	EUR	AFR
MC1R gene	R142H	rs11547464	G/a	1.00	1.00	1.00	1.00
	R151C	rs1805007	C/t	1.00	1.00	0.96	1.00
	I155T	rs1110400	T/c	1.00	1.00	1.00	1.00
	V156L	rs3212365	G/c	1.00	1.00	1.00	1.00
	R160W	rs1805008	C/t	0.96	1.00	0.94	1.00
	R163Q[b]	rs885479	G/a	0.16	0.38	1.00	1.00
	F196L	rs3212366	T/c	1.00	1.00	1.00	1.00
	T272M	rs12102534	C/t	1.00	1.00	1.00	1.00
	D294H	rs1805009	G/c	1.00	1.00	1.00	1.00
	F300F	rs3212367	C/t	1.00	1.00	1.00	1.00
	3′UTR	rs3212368	G/a	1.00	1.00	1.00	0.75
P gene	IVS5+1001[b]	rs749846	G/t	0.12	0.00	0.96	1.00
	P241R	rs2305253	G/c	1.00	1.00	1.00	1.00
	A257D	rs1050968	G/t	1.00	1.00	1.00	1.00
	R419Q	rs1800407	C/t	1.00	1.00	0.98	1.00
	L440F	rs1800408	C/g	1.00	1.00	1.00	1.00
	IVS13+113[b]	rs1900758	C/t	1.00	0.75	0.32	0.50
	H615R[b]	rs1800414	T/c	0.44	0.63	1.00	1.00

[a] Allele frequency of a major allele (written by a capital letter)
[b] SNP with large differences in frequency between populations

Linkage disequilibrium analysis and determination of diplotype groups

Linkage disequilibrium (LD) between a pair of loci was tested for SNP2–SNP4 (See Supplementary Table S1) on the same chromosome using the likelihood ratio test and the expectation–maximization (EM) algorithm [36]. LD analysis and the estimation of haplotypes were also performed using ARLEQUIN ver.3.1. The diplotype groups formed by SNP2–SNP4 were determined from the observed genotypes and haplotypes estimated by ARLEQUIN ver.3.1.

Combined genotypes

In this study, we propose a genotypic tool termed as combined genotype (CG) that is a combination of a genotype (SNP1) and a diplotype group (SNP2–SNP4). CG distribution was investigated at the interpopulation level. Significant differences in CG frequencies among six populations (JPN/KRE/CHN/MGL/EUR/AFR) were determined according to the procedure of Raymond et al. [37] and Goudet et al. [38] using ARLEQUIN ver.3.1.

Overlap rate of CG

The overlap rate of CG between a pair of populations was defined by the following expression.

$$OV(pop1-2) = \{N(pop1=2)/N(pop1)\} \times 100(\%)$$

where OV(pop1-2) was the overlap rate that indicated the degree to which CGs in *population1* overlapped with those in *population2*; *N*(pop1=2) was the number of individuals in *population1* who shared a common CG with *population2*; and *N*(pop1) was the number of individuals in *population1*.

Odds ratio

The odds ratio was calculated using CG frequencies to estimate the population origin of an individual with a particular CG. In this study, the odds ratio was calculated by converting the minimum frequency value (excluding 0) among populations to 1.00. A value of 0 was left unchanged.

Results

SNP genotyping

The allele frequencies determined by preliminary sequencing analyses are shown in Table 1. Fourteen SNPs were excluded from subsequent genotypic analyses because the allele frequencies were not very different among the study populations. PCR-APLP analysis was performed using the remaining four SNPs (See Supplementary Table S1). The PCR products exhibiting polymorphisms are shown in Fig. 1.

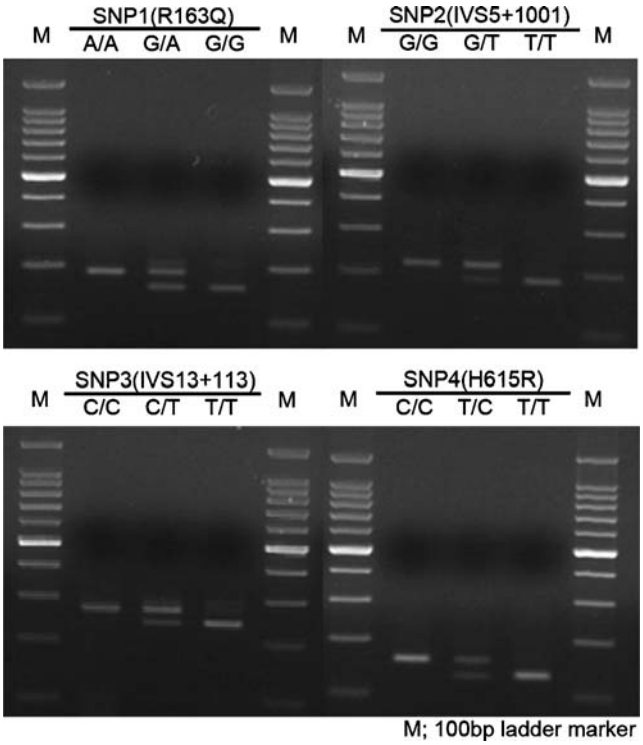

Fig. 1 SNP genotyping using the PCR-APLP method. PCR products of SNP1-SNP4 were electrophoresed in a 2% agarose gel, and it was confirmed that two specific alleles in each SNP were amplified and separated with exact reproducibility

The *P* values for HWE showed no significant difference (Table 2). The variants of SNP1, SNP2, SNP3, and SNP4 were found with relatively higher frequencies in the Asian groups (JPN/KRE/CHN/MGL) than in the EUR/AFR groups. In addition, in the Asian groups, the allele frequencies for each SNP differed significantly ($P < 0.025$, χ^2-test) between the JPN and KRE/CHN/MGL groups.

CG

The LD analysis revealed that at least one or more pairs of SNP2–SNP4 were in linkage disequilibrium ($P > 0.05$) in each population and that each pair of SNP2–SNP4 was in linkage disequilibrium in at least one or more of the six populations. This finding suggested that these three SNPs were linked with each other although LD was unlikely to be significant.

ARLEQUIN ver.3.1 estimated seven haplotypes formed by SNP2–SNP4: GCC, GCT, GTT, TCC, TCT, TTC, and TTT. In this study, 18 diplotype groups of SNP2–SNP4 were determined from the observed genotypes and haplotypes estimated by ARLEQUIN ver.3.1 (Table 3). Here, we present a new genotyping tool termed as CG, which is the combination of a genotype (SNP1) and a diplotype group (SNP2–SNP4).

Differentiation test

The differentiation test for CG frequencies between the six populations showed no significant difference ($P > 0.05$) between the three Asian populations (KRE/CHN/MGL). Therefore, we reconstituted the six populations as four populations (JPN/KCM/EUR/AFR) such that the KCM population comprised the KRE, CHN, and MGL populations. Statistical analyses were then performed for the four populations at the interpopulation level.

Overlap analysis

The overlap rate of CG among the four populations is shown in Table 4. The JPN and KCM groups had a large overlap of CGs; 98% of JPN volunteers showed CGs confirmed in the KCM group, and 79.6% of KCM volunteers showed CGs confirmed in the JPN group.

Table 2 Allele count and *P*-value for Hardy-Weinberg equilibrium (HWE) in 6 populations

SNP	allele	Allele number						*P* value for HWE[a]					
		JPN	KRE	CHN	MGL	EUR	AFR	JPN	KRE	CHN	MGL	EUR	AFR
1	G	77	2	15	36	149	102	1.000	1.000	0.636	1.000	0.064	1.000
	A	323	6	33	94	5	2						
2	G	36	2	5	30	128	78	0.663	1.000	1.000	0.488	0.209	0.710
	T	364	6	43	100	26	26						
3	T	27	2	13	33	122	68	0.607	1.000	0.135	1.000	0.728	0.231
	C	373	6	35	97	32	36						
4	T	176	5	28	97	154	104	0.565	0.428	0.672	0.741	nt[b]	nt[b]
	C	224	3	20	33	0	0						
	n	400	8	48	130	154	104						

[a] Significance level $P=0.05$
[b] Not tested because one of the two alleles was absent

Table 3 Diplotype groups formed by SNP2-SNP4

Diplotype group	Diplotypes formed by SNP2-SNP4
D1	GTT/GTT
D2	GTT/GCT
D3	GCT/GCT
D4	GCC/GCC
D5	GTT/TTT
D6	GTT/TTC
D7	GTT/TCT, GCT/TTT
D8	GTT/TCC, GCC/TTT, GCT/TTC
D9	GCT/TCT
D10	GCT/TCC, GCC/TCT
D11	GCC/TCC
D12	TTT/TTT
D13	TTT/TCT
D14	TTT/TCC, TTC/TCT
D15	TTC/TCC
D16	TCT/TCT
D17	TCT/TCC
D18	TCC/TCC

Further, the EUR and AFR groups also showed considerable overlapping of CGs (EUR: 94.8% and AFR: 96.2%). On the other hand, there were few common CGs between the Asian (JPN/KCM) and EUR/AFR groups; in particular, the JPN and EUR/AFR groups had extremely few overlapping CGs (0–3.0%).

Assignment test

In this study, 41 types of CGs were identified, and the odds ratio for each individual CG was calculated between the four populations (See Supplementary Table S2).

Discussion

Consistent with previous reports [39–42], our result showed that the variants of SNP1 and SNP4 were present with high frequencies in the Asian groups (JPN/KRE/CHN/MGL) but had low frequencies in the EUR/AFR groups (Table 2). In addition, the variants of SNP2 and SNP3 also appeared with relatively higher frequencies in the Asian groups than in the EUR/AFR groups, although the difference in allele frequencies was not as large as that observed in the case of SNP1 and SNP4.

Here, we suggest that the variants of SNP1 and SNP4 in the Asian groups might have evolved because of genetic drift and increased rapidly in frequency by positive Darwinian selection, followed by genetic admixture with the European groups. This assumption is further supported by the results of other reports [39, 43, 44]. On the other hand, in the case of SNP2 and SNP3, we hypothesize that the allele variants might have evolved somewhere in Africa and migrated to both Europe and East Asia. In this process, most of the groups that migrated to East Asia might have carried the allele variants with high frequency by chance, and genetic admixture with the European groups might have occurred after the journey. Probably, positive Darwinian selection might not have played a role in the case of SNP2 and SNP3. Hence, the allele variants of SNP2 and SNP3 appeared with higher frequency than those in the case of SNP1 and SNP4 in the EUR/AFR groups.

Furthermore, the variants of SNP1–SNP4 appeared with relatively higher frequency in the JPN group than in the KRE/CHN/MGL groups (Table 2). This might be a result of both the geographical location of Japan, which is separated from the Eurasian continent by the Sea of Japan, and its long-term closed-door policy. As a result, only the JPN group among the Asian groups might not have had sufficient opportunity for genetic exchange with the European populations.

These differences in allele frequencies between the populations definitely affect CG distribution as shown in Table 4. The result of overlap analysis suggests that the CG formed by SNP1–SNP4 can be a powerful tool for discriminating between the Asian (particularly JPN) and EUR/AFR groups, although it is not so effective for discriminating between the JPN and KCM groups or between the EUR and AFR groups.

In this study, 41 types of CGs were identified (see Supplementary Table S2). Here, we assume that the difference in odds ratio between the populations indicates the difference in the degree to which a particular individual belongs to a population. Therefore, it is assumed that the population origin of an individual with a particular type of CG could be estimated from the list of populations using the odds ratio data. For example, with regard to type C1 in Supplementary Table S2, the odds ratio is highest in the EUR group (2.53), followed by the AFR group (1.00), and 0 in both the JPN group and KCM groups. On the basis of this result, we estimate that individuals with type C1 would belong primarily to the EUR group, secondarily to the AFR group, and to a limited extent to the JPN and KCM groups.

Table 4 Overlap rate (%) of CG among 4 populations

Population 1	Population 2			
	JPN	KCM[a]	EUR	AFR
JPN		98.0	3.0	0.0
KCM[a]	79.6		4.3	3.2
EUR	1.3	23.4		94.8
AFR	0.0	38.5	96.2	

[a] Total of KRE, CHN, and MGL

Similarly, in other 40 types of CGs, the population origin of an individual is assumed to be estimated with ranking from the four listed populations as shown in Supplementary Table S2.

In addition, since a CG is the combination of both a genotype and a diplotype group, easy identification is possible by merely genotyping each specimen for SNP1–SNP4. Further, particularly in European people, we realized that the four SNPs were not associated with skin, hair, and iris color at both a single locus and CG levels (data not shown). The CG method is optimized for discriminating between the Asian groups and EUR/AFR groups, and the population-specific markers for the EUR and AFR groups should be included for the general estimation of the population origin of an individual. Therefore, we plan to examine the following four SNPs partly described in Shriver et al. [8] as the markers: *DARC* (rs2814778), *F13B* (rs6003), *TYR* (rs1042602), and *GUCY2D* (rs2816).

In summary, our findings show that the CG formed by SNP1–SNP4 would be effective for estimating the population origin of an individual with ranking from a list of four populations (JPN/KCM/EUR/AFR). This method is especially suitable for discriminating between the Asian (particularly JPN) and EUR/AFR groups. In addition to the four SNPs in this study, we plan to examine four other variants that differ in allele frequencies between European and African populations. By combining these new variants together with our SNPs, the CG approach can serve as an extremely powerful tool for estimating the population origin of an individual.

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