論文の内容の要旨

論文題目　Identification of genetic polymorphisms related to skin reflectance in the Japanese population

（日本人における皮膚色関連遺伝子多型の同定）

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With the interface between the body and the environment, the functions of the skin may arguably be regarded as adaptations to the different environments that our ancestors were exposed to, shaping the geographical variation in skin characteristics, including pigmentation in modern humans. The apparent geographical variation in skin pigmentation has attracted both public and academic attention, and has been studied in many research areas such as evolutionary biology, clinical medicine, health science, physiology, and molecular genetics. Most studies on the genetic basis of human skin pigmentation, however, have focused on people of European ancestry, and only a few studies have focused on Asian populations. Therefore, this study investigates the association of skin reflectance and freckling with genetic polymorphisms in the Japanese population.

DNA samples were obtained from a total of 653 Japanese individuals (ages 19-40) residing in Okinawa. Skin reflectance was measured using a spectrophotometer and freckling status was determined for each individual. A lightness index (L*) of the inner upper arm and freckling status were not correlated with age, BMI, or ancestry (Ryukyuans or Main Islanders of Japan).

In Chapter 1, the association of skin reflectance and freckling with polymorphisms of the *Melanocortin 1 Receptor* (*MC1R*) is examined. The *MC1R* is located on human chromosome 16q24.3, and it encodes the protein MC1R, a G protein-coupled seven transmembrane receptor for α-melanocyte simulation hormone (α-MSH). When α-MSH binds to MC1R, subsequent activation of

adenylate cyclase facilitates accumulation of cAMP inside the melanocyte. This accumulation ultimately promotes eumelanin (dark pigment) synthesis. Therefore, MC1R activation plays a key role in skin pigmentation because it results in a switch from pheomelanin (light and immature pigment) synthesis to eumelanin synthesis. Among the 10 nonsynonymous variants that were identified by direct sequencing of the coding region of *MC1R*, two variants were most common —R163Q and V92M—with the derived allele frequencies of 78.6% and 5.5%, respectively. The others were observed at low frequencies (<5%) and hence, are defined as "rare variants." As shown in the largest circle with many branches in the haplotype network (Figure 1), the majority of the estimated haplotypes had the 163Q allele. Tajima's D was calculated as -1.66 with *P*-value of 0.015, which suggested a deviation from neutrality and the possibility of purifying or positive selection on *MC1R*.

Stepwise multiple regression analysis showed that the 163Q allele and the presence of nonsynonymous rare variants were significantly associated with an increase in sex-standardized skin lightness (L* of CIELAB) of the inner upper arm, designated ZuaL* (Figure 2). Relative to the 92V allele, the 92M allele was significantly associated with increased odds of freckling. R163Q was reported as an example of East Asian selective sweep, although its functional significance was then unknown. This study thus shows the effect of the163Q allele on skin reflectance values. This association indicates that light-toned skin may have been subject to positive selection in East Asian people.

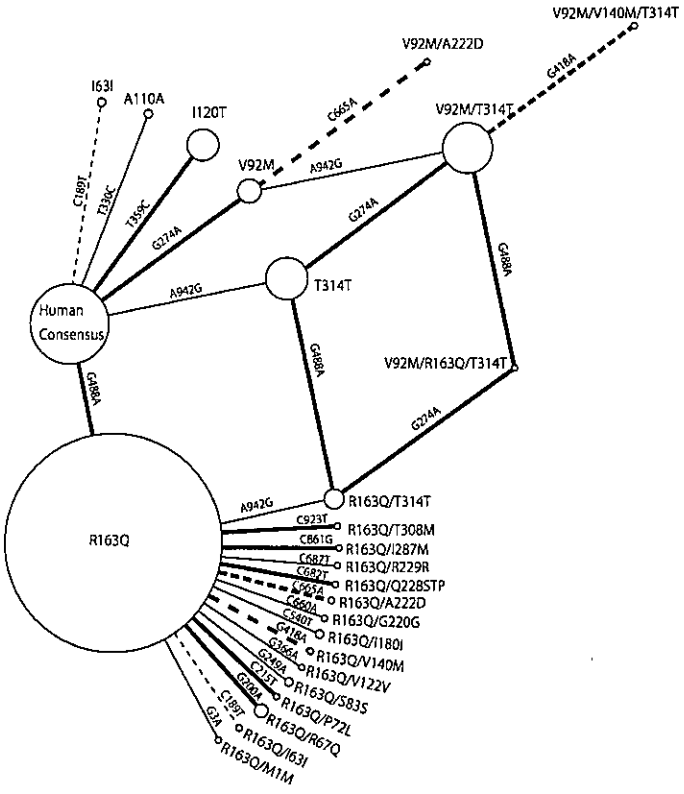

Figure 1 *MC1R* haplotype network based on the estimated haplotypes of 1306 chromosomes evaluated in this study.

Each circle represents a haplotype with its amino acid change(s) annotated by or in the circle. The size of each circle reflects frequency of the haplotype. Base changes are annotated along the lines connecting circles, which are broad for nonsynonymous substitution. The three haplotypes estimated with the probability of 0.5 with R163Q are indicated by dotted lines.

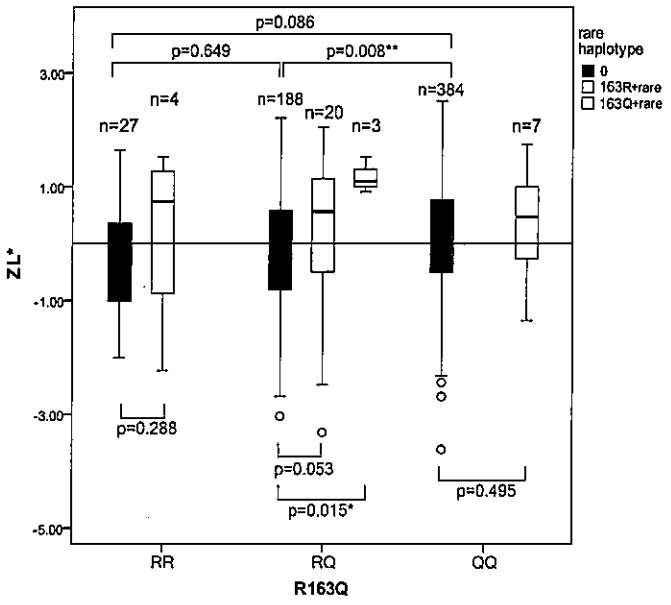

Figure 2 Boxplot of L* (lightness) categorized by R163Q genotype and clustered by rare haplotypes. "163R+rare" or "163Q+rare" means the rare variant is located on the same haplotype as the 163R or 163Q allele, respectively. "0" means no rare variant is present. The bottom of the box is the 25th percentile, the center line is the median, and the top of the box is the 75th percentile. *P*-values were determined using the Mann-Whitney *U* test.

In Chapter 2, single nucleotide polymorphisms (SNPs) that had significant associations with skin pigmentation and freckling were identified through the analyses in the discovery study (86 females and 209 males), in the replication study (203 females and 155 males), and in the total sample.

In the discovery study, SNPs were selected if they satisfied either or both of the following criteria: 1) associations of the SNPs with skin pigmentation in people of European descent were reported, 2) positive selective sweep on the SNP or its adjacent region in the sample of East Asians were suggested by the previous studies in population genetics. A total of 59 SNPs were genotyped using TaqMan assays, a DigiTag2 assay, or direct sequencing. Two SNPs in *Oculocutaneous Albinism II* (*OCA2*), rs12442916 (rs: reference SNP number from dbSNP, NCBI), and rs7465330 had *P*-values smaller than 0.01for the correlation with ZuaL*, and hence were further examined in the replication study together with a nonsynonymous polymorphism (rs1800414) located near these two SNPs. Only rs6058017 in *Agouti Signaling Protein* (*ASIP*) showed a significant ($P<0.05$) association with freckling, and the ancestral allele had significantly higher odds for freckling relative to the derived allele.

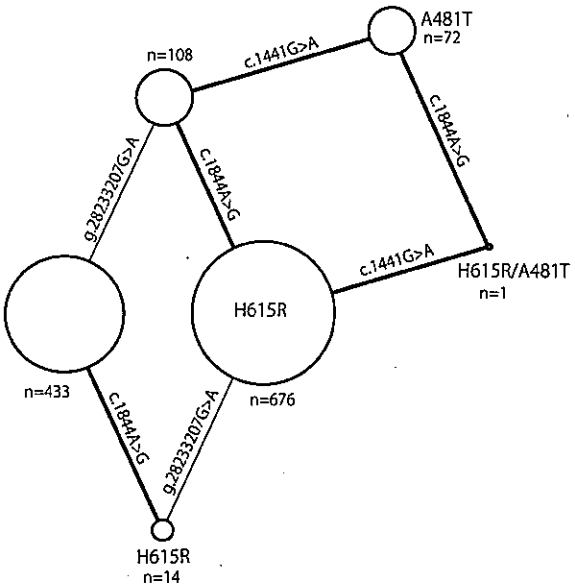

Figure 3 *OCA2* haplotype network based on the estimated haplotypes of 1304 chromosomes evaluated in this study.

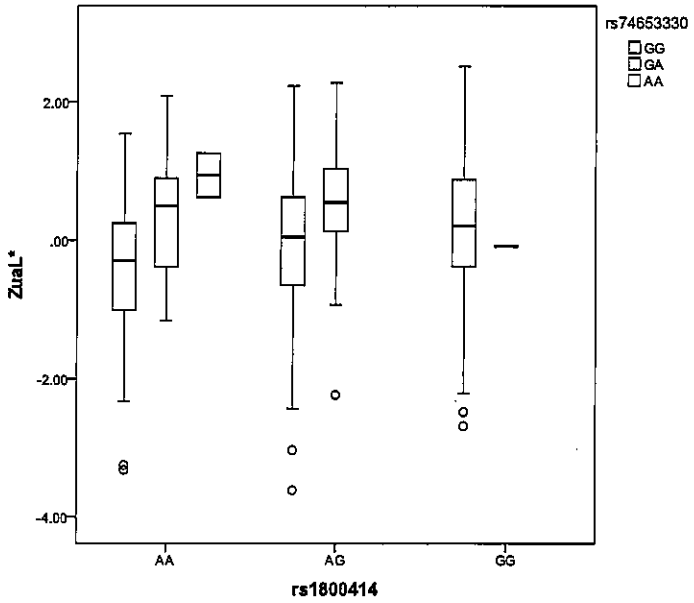

Figure 4 Boxplot of L* (lightness) categorized by R163Q genotype and clustered by rare haplotypes.

In the replication study, the correlations of the three SNPs in *OCA2* with ZuaL* were confirmed. After merging the discovery and the replication studies, stepwise multiple regression analyses showed that rs7465330 and rs1800414 had significant associations with ZuaL* and suggested that the derived alleles would increase the lightness in an additive manner. A haplotype network of these three SNPs (Figure 3) showed that the derived alleles of rs74653330 (A481T) and of rs1800414 (H615R) had occurred on the derived allele of rs12442916 (g.28233207G>A), and they do not share any haplotype except for a low-frequency haplotype observed in only one chromosome. This explained the apparent association of rs12442916 and skin reflectance, and confirmed the independent effects of A481T and H615R on the lightness of skin among Japanese people (Figure 4). *OCA2* has been reported as one of the genes that had signatures of positive selection in Asia, and therefore, the results of this chapter also suggest that the light-toned skin was subject to selection in East Asia.

To summarize, the 163Q allele of *MC1R* and the 481T and 615R alleles of *OCA2* were associated with the lightness of skin reflectance, and the 92M allele of *MC1R* and the ancestral allele of rs6058017 in *ASIP* showed higher odds for freckling. These results explain part of the difference in constitutive skin pigmentation between Asian and non-Asian populations as well as the north to south gradient in pigmentation across Asia. Association studies on admixed populations or comparative studies in allele frequencies among different populations are required to further elucidate the evolution of skin pigmentation among peoples of Asia.