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Linkage and association scan for tanning ability in an isolated Mongolian population

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Tanning ability is important, because it represents the ability of the skin to protect itself against ultraviolet (UV) radiation. Here, we sought to determine genetic regions associated with tanning ability. Skin pigmentation was measured at the outer forearm and buttock areas to represent facultative and constitutive skin color, respectively. In our study population consisting of isolated Mongolian subjects, with common histories of environmental UV exposure during their nomadic life, facultative skin color adjusted by constitutive skin color was used to indicate tanning ability. Through linkage analysis and family-based association tests of 345 Mongolian subjects, we identified 2 potential linkage regions regulating tanning ability on 5q35.3 and 12q13.2, having 6 and 7 significant single nucleotide polymorphisms (SNPs), respectively. Those significant SNPs were located in or adjacent to potential candidate genes related to tanning ability: *GRM6, ATF1, WNT1,* and *SILV/Pmel17*. [BMB reports 2011; 44(11): 741-746]

INTRODUCTION

Most of the apparent acute clinical effects of ultraviolet radiation (UVR) consist of sunburn and tanning, while chronic exposure to UVR can cause photoaging and skin cancer (1). Tanning, however, can provide protection against future UV irradiation (2). Therefore, tanning ability is important, because it represents the capacity of the skin to protect itself against UVR. In a previous study, low tanning ability, along with other pigmentary traits (e.g., fair skin color and hair color), were shown to be risk factors for skin cancer (3).

The difference between the skin color of a sun exposed area (i.e., facultative skin color, FSC) and that of a sun protected area (i.e., constitutive skin color, CSC) has been used to evaluate the extent of tanning. FSC designates increases in melanin pigmentation above CSC after tanning (4). The amount of tanning (i.e., the difference between FSC and CSC) varies among individuals after the same amount of UV exposure, thereby revealing differences in tanning ability. As our study population was comprised of isolated Mongolian subjects who lived a nomadic life and had histories of environmental UV exposure in common, FSC adjusted by CSC would be indicative of tanning ability. Therefore, we conducted a gene mapping study to search for potential tanning ability related genes using adjusted FSC as a basis.

Tanning ability belongs to a group of pigmentary traits that include eye, hair, and skin colors. To date, 6 genome-wide association studies (GWASs) for pigmentary traits have been conducted and have identified 11 genes related to pigmentation (5-10). Furthermore, based on comparative genomics, there are 378 loci (171 cloned genes and 207 uncloned genes) which influence pigmentation in mice and their human and zebrafish homologues which are available from the European Society for Pigment Cell Research Web site (www.espcr.org/micemut/). However, self-reported questionnaires instead of objectively based measurements were used to assess pigmentary traits, including tanning ability, in most previous GWAS studies. As self-reported tanning ability is not synonymous with objectively measured tanning ability, the question of which genes are responsible for variations in tanning ability that are objectively measured among individuals has not been clearly answered.

In this study, to determine genetic regions associated with objectively based measures of tanning ability, we conducted a genome-wide scan linkage analysis of tanning ability. In addition, family-based association tests were used for finer-scale genetic mapping in selected regions with potential linkage peaks.

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Table 1. Linkage regions from genome-wide linkage scan for MI in outer forearm

Chromosome (location)	Maximum LOD score	Nearest marker	Cytogenetic region	Empirical P value	Locus-specific heritability
5 (205)	1.74	D5S2030	5q35.3	0.0017	0.59
12 (71)	1.69	D12S1707	12q13.2	0.0018	0.54

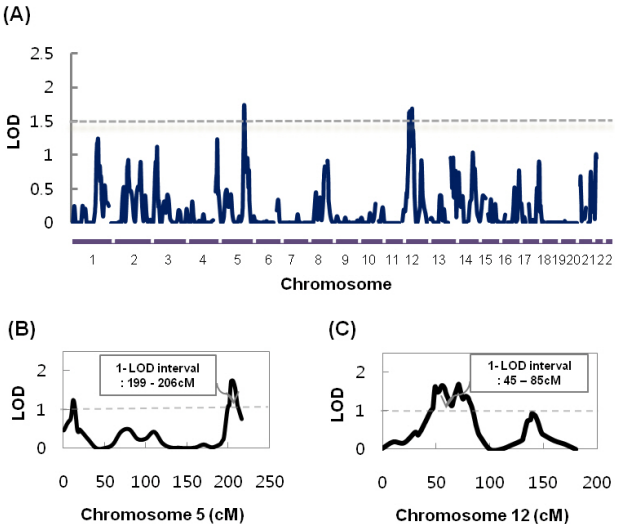

Fig. 1. Multipoint linkage results for tanning ability. (A) Genome wide linkage scan results for tanning ability across 22 autosomes. (B) Chromosome 5 (1-LOD score unit supports interval region range of 199 cM to 206 cM). (C) Chromosome 12 (1-LOD score unit supports interval region range of 45 cM to 85 cM).

RESULTS

Participant characteristics

For this study, 59 families composed of 345 individuals were assessed. The majority (58%) of participants were female and the mean age of all subjects was 30.4 years. The mean (standard deviation, SD) melanin index (MI) values in the outer forearm of male and female subjects were 460.6 (86.9) and 398.5 (80.5), respectively, and were significantly different (P value $<$ 0.0001). The MI in the buttock indicated constitutive skin color was lighter than that in the outer forearm (P value $<$ 0.0001). In the buttock, the MI of females was lower than that of males, and the difference was statistically significant (P value $<$ 0.0001).

Genome wide linkage analysis

We conducted genome wide linkage analysis for tanning ability using the variance component method across 22 autosomes. As shown in Table 1 and Fig. 1A, we found 2 linkage regions related to tanning ability with a logarithm of odds (LOD) score of $>$ 1.5. We identified the highest linkage locus located on 5q35.3 with a LOD score of 1.74 (empirical P value = 0.0017). The locus specific heritability was 0.59 and the nearest marker was D5S2030. The second peak was at the 12q13.2 locus, and its LOD score was 1.69. For that peak, the locus specific heritability was 0.54 and the nearest marker was D12S1707 (empirical P value = 0.0018). Fig. 1B and 1C show a more detailed graphic display of the 2 candidate linkage chromosomes. On chromosome 5, the linkage support region (1-maximum LOD score interval) was narrow and ranged from 199 cM to 206 cM (Fig. 1B). On chromosome 12, the linkage support interval spanned ~40 cM, from 45 cM to 85 cM (Fig. 1C).

Family based association analysis

We initially identified the 2 candidate linkage regions from a genome wide linkage scan (Fig. 1). Next, we conducted an association fine mapping study using additional single nucleotide polymorphism (SNP) markers under the 2 identified linkage regions. After filtering QC, the number of SNPs used on chromosomes 5 and 12 were 793 and 8,583, respectively. Table 2 shows results of a family based association test in identified linkage regions. For the statistical problem of multiple comparisons, we used a strict Bonferroni adjusted P value of significance threshold (Bonferroni adjusted P value $<$ 0.05). On chromosome 5, the strongest association was rs3733915 SNP in the *GRM6* gene on 5q35.3 (FBAT P value = 1.86×10^{-7}). Of the other candidate 5 SNPs, 4 SNPs (rs17664029, rs4700971, rs4700752, and rs12186577) except for rs7727323, were located within 50 kb of the *GRM6* gene. On chromosome 12, we discovered the 7 significant SNPs (rs10783388, rs10877157, rs12809834, rs981494, rs11613963, rs11170624, and rs10876477) associated with tanning ability. The strongest associated SNP was rs10783388 in the *ATF1* gene on 12q13.12 (FBAT P value = 9.60×10^{-7}). The second most significant SNP was rs10877151 on 12q14.1 (FBAT P value = 9.72×10^{-7}), and was located in an intergenic region. Fig. 2 show a regional association LD plot for novel loci rs3733915 near *GRM6* on chromosome 5 and rs10783388 near *ATF1* on chromosome 12, respectively.

DISCUSSION

Human skin color is influenced by various factors, including sex. Adult females tend to have lighter skin color than males of the same age (11-16). In our study, the MI in both the outer forearm and buttock areas of females was lower than that of

Table 2. Significant SNP markers in family based association test within linkage peak region

SNP	Chromosome (location, bp)	Cytogenetic region	Minor allele (MAF)	FBAT P value	Bonferroni adjusted P value	FDR Q value
rs3733915	5 (178,339,727)	5q35.3	G (0.09)	1.86×10^{-7}	1.48×10^{-4}	1.00×10^{-4}
rs17664029	5 (178,300,702)	5q35.3	G (0.21)	2.21×10^{-6}	1.75×10^{-3}	9.00×10^{-4}
rs4700971	5 (178,315,216)	5q35.3	A (0.44)	6.76×10^{-6}	5.36×10^{-3}	1.30×10^{-3}
rs4700752	5 (178,315,473)	5q35.3	T (0.44)	6.76×10^{-6}	5.36×10^{-3}	1.30×10^{-3}
rs12186577	5 (178,292,595)	5q35.3	C (0.10)	1.10×10^{-5}	8.70×10^{-3}	1.70×10^{-3}
rs7727323	5 (177,712,789)	5q35.3	G (0.11)	3.59×10^{-5}	2.85×10^{-2}	4.70×10^{-3}
rs10783388	12 (49,467,966)	12q13.12	T (0.41)	9.60×10^{-7}	8.24×10^{-3}	3.70×10^{-3}
rs10877157	12 (57,331,752)	12q14.1	C (0.41)	9.72×10^{-7}	8.34×10^{-3}	3.70×10^{-3}
rs12809834	12 (28,224,773)	12p11.22	G (0.42)	1.98×10^{-6}	1.70×10^{-2}	3.70×10^{-3}
rs981494	12 (28,217,116)	12p11.22	C (0.48)	2.04×10^{-6}	1.75×10^{-2}	3.70×10^{-3}
rs11613963	12 (39,416,187)	12q12	A (0.11)	2.91×10^{-6}	2.50×10^{-2}	3.70×10^{-3}
rs11170624	12 (52,316,505)	12q13.13	T (0.25)	2.95×10^{-6}	2.53×10^{-2}	3.70×10^{-3}
rs10876477	12 (52,339,513)	12q13.13	T (0.26)	3.02×10^{-6}	2.59×10^{-2}	3.70×10^{-3}

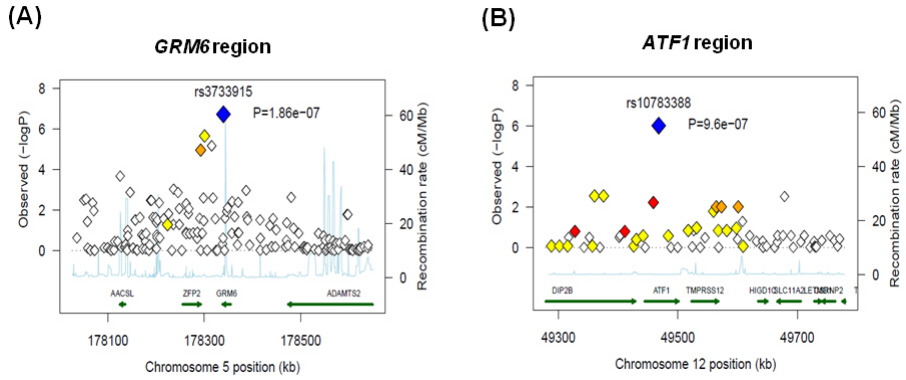

Fig. 2. Regional plots of the strongest association chromosomes 5 and 12. (A) *GRM6* gene region on 5q35.3 and (B) *ATF1* gene region on 12q13.12 are plotted using P value (as $-\log_{10}$ value) as a function of genomic position (Build 36). In each plot, the strongest SNP (rs3733915 and rs10783388) is represented by a blue diamond. Local LD structure is reflected by the plotted estimated recombination rates (from HapMap). The color scheme of the diamond about LD patterns: $r^2 < 0.2$; white, $0.2 \leq r^2 < 0.4$; yellow, $0.4 \leq r^2 < 0.7$; orange, $r^2 \geq 0.7$; red.

males and these results are consistent with those of previous studies. Concerning the difference between CSC and FSC, the MI in the buttock was much lighter than that in the outer forearm, reflecting the tanning effect of UVR ($P < 0.0001$).

Prior to undertaking a gene mapping study, heritability analysis has been used to assess genetic evidence of interesting traits (17). Our 2 genome wide linkage peaks show locus specific heritability on 5q35.3 of 0.59 and on 12q13.2 of 0.54 (Table 1). To the best of our knowledge, there have been no previous reports concerning heritability of tanning ability; previously, only heritability of human skin color was reported and ranged from 0.55 to 0.83 (12, 18). Our results show that tanning ability, as measured by FSC adjusted by CSC is moderately affected by genetic factors, and heritability of that trait is comparable to that for human skin color.

To date, there have been no reports concerning linkage analysis of tanning ability or other pigmentary traits. Previously, 6 GWAS for pigmentary traits identified 11 genes related to pigmentation: *TYR* (11q14.3), *TYRP1* (9P23), *OCA2* (15q12∼q13.1), *SLC45A2* (5p13.2), *SLC24A5* (15q21.1), *MC1R* (16q24.3), *ASIP* (20.q11.22), *KITLG* (12q21.32), *SLC24A4* (14q32.12), *IRF4* (6p25.3), and *TPCN2* (11q13.3) (5-10). Here, our linkage analysis revealed additional peaks in 2 other chromosome regions (Table 1 and Fig. 1).

We found a novel suggestive candidate region with an LOD score of 1.74 on 5q35.3. In that region, the most significant SNP (rs3733915) was located in 3′-UTR of *GRM6* (metabotropic glutamate receptor 6). Recently, a signal transduction pathway initiated by metabotropic glutamate receptor 6 was revealed to end with the opening of the transient receptor potential (TRP) M1 cation channel (19). In human neonatal epidermal melanocytes, expression of *TRPM1* is associated with melanin content, implying that its function is critical to normal melanocyte pigmentation (20). Others have reported that a *TRPM1* mutation causes leopard complex (LP) spotting in a horse model (21, 22). Therefore, we suggest that *GRM6* is a candidate gene for the control of

tanning ability.

We detected a candidate region with an LOD score of 1.69 and with 7 significant SNPs on 12q13.2. Among them, the most significant SNP (rs10783388) was located in intron 2 of *ATF1* (activating transcription factor 1). *MITF* (Microphthalmia-associated transcription factor) has been termed the master gene for melanocyte survival and is a key factor regulating the transcription of the major melanogenic proteins, such as tyrosinase (*TYR*) and tyrosinase-related protein (*TRP*) (23). The regulation of *MITF* expression is under control of several transcription factors including *ATF1* (24), which is known to be activated after UVR in a melanoma cell line (25). In addition, previously well-known color genes *WNT1* (wingless-type MMTV integration site family, member 1) and *SILV/Pmel17* (silver homolog) are located near the significant SNPs. The Wnt pathway is also known to regulate the expression of *MITF*, while Pmel17 is a major structural protein of melanosomes, which is a melanocyte specific organelle where melanin biosynthesis occurs (26). In a recent study, *SILV/Pmel17* was revealed as the gene with the most increased expression, whereas *WNT1* showed decreased expression after *in situ* exposure of human skin to repetitive sub-erythemal UVR (27). UV induced changes in gene expression and protein activity imply that those types of genes engage in UV induced effects, including tanning. Especially, as exposure to repetitive sub-erythemal UVR resembles the situation in environmental UV exposure, *WNT1* and *SILV/Pmel17* seem to be more responsible for the tanning response. These UV responsive genes, *ATF1*, *WNT1*, and *SILV/Pmel17*, also would be candidate tanning control genes.

To verify the previously reported 11 genes associated with human pigmentary traits, we conducted an association study using SNPs near the regions of those genes. However, we found no significant SNPs associated with tanning ability (data not shown). Regardless, our results do show unique regions of potential linkage, significant SNPs, and candidate genes that have not been previously reported in GWAS concerning human pigmentary traits. The uniqueness may be due to ethnic differences in subject populations. For example, rs3733915, the most significant SNP in our study, is essentially monomorphic in other European and African populations (see http://www.ncbi.nlm.nih.gov/projects/SNP/). It seems that our findings have identified novel genes regulating tanning ability; most notably within an Asian population.

Moreover, our objective measurements of tanning ability, which were expressed as adjusted FSC, provide our study an advantage over studies using other measures of tanning ability. First, in the case of self-reported tanning ability obtained via a questionnaire, there could be a problem of recall bias. Second, adjusted FSC reflecting chronic UV exposure is more indicative of the ability of the skin to protect itself against UVR than pigmentation increases after short term experimental exposure to UVR.

In conclusion, we identified 2 potential linkage regions regulating tanning ability on 5q35.3 and 12q13.2 and 6 and 7 significant SNPs on respective linkage regions located in or adjacent to the loci of the potential candidate genes of tanning ability. Further functional studies of these potential candidate genes will be needed to elucidate tanning responses to UVR. Our results contribute to furthering the study of UV photobiology.

MATERIALS AND METHODS

Subjects

In 2007, 750 subjects were sampled in Dashbalbar, Dornod Province, Mongolia. To obtain powerful linkage detection, we only included extended, large pedigrees of individuals. As a result, the pedigrees used in this study were from 59 families composed of 345 family members. Pedigree structure information was determined by personnel interviews and was reconfirmed by genotyping markers. This study abided by the principles of the Declaration of Helsinki, was approved by the institutional review board of Seoul National University, and informed consent was obtained from all participants.

Skin pigmentation measurement

We measured skin pigmentation using a Mexameter MX18® (Courage and Khazaka, Köln, Germany), which is based on light absorption and reflection. The MI value for each measurement site was represented as a ratio of the quantity of light emitted from the probe and that absorbed by the skin in the range of 0 to 999. A low MI value indicates a lighter skin color. To assess the tanning effects related to UVR, both the outer forearm and buttock areas of each subject were measured. The outer forearm is well known as a sun-exposed area, whereas the buttock is indicative of a sun-unexposed area. To adjust FSC to CSC, the buttock MI value was used as a covariate when analyzing the MI value in the outer forearm area.

Genotyping

The processes of genotyping and error checking were described in previous studies (28, 29). In short, we extracted leukocyte DNA from all subjects, and all samples were genotyped using a 1039 short tandem repeat microsatellite marker platform. We checked for genotyping errors such as Mendelian (0.13%) and non-Mendelian errors (0.26%) and removed them. In addition, SNP markers were also genotyped using the Illumina Human 610-Quad Beadchip platform in subject subsamples. For quality control (QC) of SNP markers, we only considered markers with high call rates ($\geq$ 99%), low error rates ($\leq$ 1%), and minor allele frequencies ($\geq$ 0.01).

Statistical analysis

To evaluate the normal distribution of MI values in the outer forearm, we used the Shapiro-Wilk test; its distribution followed a normal distribution (Shapiro-Wilk P value = 0.052). SAS, version 9.1 was used for basic descriptive analysis. Independent *t* tests were used to test the differences between male and female characteristics. We performed variance component based link-

age analysis using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package (30), and adjusted confounding effects by including significant covariates such as age, sex, age by sex, age^2, and buttock MI in the polygenic model. For multipoint linkage analysis, multipoint identical-by-descent-matrices were calculated using the Loki package. We performed 10,000 simulations, and obtained the empirical P value.

For fine mapping in the presence of linkage, we analyzed the family based association using FBAT-GEE of the PBAT tool in Helixtree software, version 6.4 (Golden Helix Inc., Bozeman, Montana, USA). In this case, the null hypothesis of no association and presence of linkage were appropriate (31). We hypothesized an additive genetic model. To resolve the multiple comparison problems, we calculated the Bonferroni adjusted P value and Q value for control of false discovery rates.

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