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Genome-wide association analysis of 17,019 Korean women identifies variants associated with facial pigmented spots

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ABSTRACT

Variation in skin pigmentation can be affected both by environmental factors and intrinsic factors such as age, gender, and genetic variation. Recent genome-wide association studies (GWASs) revealed that genetic variants of genes functionally related to a pigmentation pathway were associated with skin pigmentary traits. However, these GWAS focused on populations with European ancestry and only a few studies have been performed on Asian populations, limiting our understanding of the genetic basis of skin pigmentary traits in Asians. To evaluate the genetic variants associated with facial pigmented spots, we conducted a GWAS analysis of objectively measured facial pigmented spots in 17,019 Korean women. This large-scale GWAS identified several genomic loci that were significantly associated with facial pigmented spots (five previously reported loci and two previously unreported loci to our knowledge), which were detected by UV light; *BNC2* at 9p22 (rs16935073; *P*-value = 2.11×10^{-46}), *PPARGC1B* at 5q32 (rs32579; *P*-value = 9.04×10^{-42}), 10q26 (rs11198112; *P*-value = 9.66×10^{-38}), *MC1R* at 16q24 (rs2228479; *P*-value = 6.62×10^{-21}), *lnc01877* at 2q33 (rs12693889; *P*-value = 1.59×10^{-11}), *CDKN2B-AS1* at 9p21 (rs643319; *P*-value = 7.76×10^{-9}), and *MFSD12* at 19p13 (rs2240751; *P*-value = 9.70×10^{-9}). Further functional characterization of the candidate genes needs to be done to fully evaluate their contribution to facial pigmented spots.

INTRODUCTION

The skin is the outermost layer of the human body and performs various functions in response to the external environment, such as protecting the body from harmful substances, aiding in perception of different sensations, and regulating body temperature (Dąbrowska et al., 2018). Proper functioning of the skin is essential for protecting the body against diseases and maintaining attractiveness (Foo et al., 2017). The types of skin pigmentations are categorized into constitutive skin pigmentation that determines the basal skin color in the absence of external environmental stimuli, and facultative skin pigmentation, such as freckles and facial pigmented spots, that affect the color in response to an exposure to stimuli such as ultraviolet (UV) rays (Del Bino et al., 2018; Shekar et al., 2005). Pigmentation levels vary greatly between and within human populations. Even when exposed to a similar environment, individuals of the same ancestry exhibit a wide spectrum of facultative skin pigmentation, suggesting that pigmented spots might be affected by genetic variants in genes involved in the process of pigmentation.

The genetic factors involved in skin pigmentary traits are not fully understood. Several studies have been conducted on candidate genes to identify such factors, in particular, polymorphisms of several genes, including *BNC2*, *UGT1A8*, *IRF4*, and *POMC* in European and American populations (Jacobs et al., 2013; Nan et al., 2009; Praetorius et al., 2013). Owing to the polygenic inheritance of skin pigmentary traits, several common genetic variants act in a probabilistic rather than deterministic fashion. Genome-wide association studies (GWASs), a systematic and powerful genetic approach for discovering hundreds or thousands of genetic loci, have been conducted to identify several genes, such as *MC1R*, *SLC24A5*, *SLC45A2*, and *BNC2*, associated with skin pigmentary traits, such as the tanning

response (Nan et al., 2009; Shido et al., 2019; Visconti et al., 2018), skin spots (Endo et al., 2018; Jacob et al., 2015), and constitutive skin pigmentation (i.e. skin color; Adhikari et al., 2019; Crawford et al., 2017; Liu et al., 2015; Peng et al., 2019). However, these studies were largely concentrated on populations with European ancestry and only a few studies have been performed in Asian populations, mainly in the Japanese population (Endo et al., 2018; Shido et al., 2019). Further studies in diverse populations are needed to discover new genetic variants or to identify causal genes regulating skin pigmentation.

In this study, data from 17,019 Korean women were used to conduct GWAS analysis, using the Illumina Global Screening Array MD BeadChip to identify genetic variants associated with facial pigmented spots and to validate the genetic effects of the variants. The measurement of facial pigmented spots was conducted using the Janus 3 system (PIE Inc., Suwon, Korea) which is one of the most widely used image analysis devices in the field of skin research in Korea (Goo et al., 2015; Kim et al., 2016; Lee et al., 2016; Leem et al., 2020; Sim et al., 2014).

RESULTS

Skin pigmentation measurements

The characteristics of the study subjects are presented in Table 1. The age distribution of the study subjects was similar between the discovery and validation stages, with an average age of 45.96 and 46.46 years, respectively. The distribution of skin measurements is shown in Supplementary Figure 1a. The facial pigmented spots of study subjects, detected by UV light, were similar for the discovery and validation stages, with an average value of 35.45 and 35.74, respectively. The facial pigmented spots of study subjects, detected by polarized light, were also similar with an average value of 27.26 for the discovery stage and 27.47 for the validation stage. Facial pigmented spots detected by both UV light and polarized light were highly correlated (Supplementary Figure 1b). The amount of skin pigmentation increased with age similarly in both the discovery and validation samples with high correlation values as shown in Supplementary Figures 2 and 3 (R^2 = 0.48 - 0.50).

Genome-wide association analysis of facial pigmented spots

In the discovery stage, a total of 13,350 Korean women were genotyped using a single nucleotide polymorphism (SNP) microarray chip. After applying the quality control (QC) criteria of genotype data, 366,864 genetic markers from 11,079 samples were used for genome-wide association analysis. A quantile-quantile (Q-Q) plot was used to test the validity of the distributional assumption for the GWAS datasets. The Q-Q plot of the GWAS stage of facial pigmented spots, detected by both UV light and polarized light, showed lambda values of 1.028 and 1.033, respectively, indicating no evidence of genomic inflation of the test statistic and an enrichment of significance of this study (Supplementary Figure 4a and 4b).

A total of five and seven loci showed Bonferroni-corrected levels of associations with facial pigmented spots, detected by UV light and polarized light, respectively (*P*-value < 1.32×10^{-7}; Figure 1, Supplementary Tables 1 and 2).

Validation of significant loci

In the validation stage, a total of 7,485 Korean women were genotyped using SNP microarray chips. After applying the QC criteria of the genotype data, 369,878 genetic markers of 5,940 samples were used for further statistical analysis. The Q-Q plot was used to test the validity of the distributional assumption for genome-wide level testing (Supplementary Figure 4c and 4d). The genetic effects of lead SNPs of associated genomic regions were validated in validation and meta-analysis (Table 2).

In the meta-analysis, several genomic loci at 9p22, 5q32, 10q26, 16q24, and 19p13, showed genome-wide significant association with facial pigmented spots, detected by both UV light and polarized light (Table 2). The genetic effects of 9p22, 5q32, 10q26, 16q24, and 19p13 were represented by rs16935073 of *BNC2*, rs32579 of *PPARGC1B*, rs11198112, rs2228479 (p.Val92Met) of *MC1R*, and rs2240751 (p.Tyr182His) of *MFSD12* with *P*-values of 2.11×10^{-46}, 9.04×10^{-42}, 9.66×10^{-38}, 6.62×10^{-21}, and 9.70×10^{-9}, respectively, for facial pigmented spots detected by UV light. These associations were also significant for facial pigmented spots detected by polarized light. Of note, among the significant SNPs, previously unreported association signals to our knowledge near *lnc01877* and *CDKN2B-AS1* were identified for facial pigmented spots detected by both UV light and polarized light. The lead SNPs (rs12693889 and rs643319) of the two loci were located in intronic regions of *lnc01877* and *CDKN2B-AS1*, respectively. Regional plot and linkage disequilibrium (LD) pattern of genomic regions for the two loci are presented in Supplementary Figure 5 and 6.

DISCUSSION

Skin pigmentation induced by extrinsic factors, such as exposure to UV rays and non-use of sunscreen, is known as facultative skin pigmentation, while constitutive skin pigmentation is determined by intrinsic factors, such as genetic composition in individuals (Parra, 2007; Pezic et al., 2013). Most studies evaluating skin pigmentary traits have been conducted on European and/or African populations, and relatively few have examined Asian populations (Hillebrand et al., 2001; Pavan and Sturm, 2019). And it remains unclear whether genetic variations determining skin pigmentation are shared among different populations or distinct genetic variants affect skin pigmentary traits (Beleza et al., 2013). For a deeper understanding of the role of genetic effects in skin pigmentary traits, studies in diverse populations are still needed. To investigate genetic markers for facial pigmented spots in a Korean population, GWAS was conducted in approximately 17,000 individuals. When performing association analyses with an adequately sized sample, GWAS can identify variants for traits from genome-wide statistical fluctuation (McCarrol and Hyman, 2013). In this regard, the number of samples included in this study was sufficient to identify multiple significant genetic variants related to facial pigmented spots. These results also highlight the value of the image-based method used in this study for evaluating pigmentation objectively.

In this study, we investigated the association of genetic variants with facial pigmented spots in a Korean population. The genetic effects of lead SNPs identified in the present study were consistent with those from previous Japanese studies on facial skin pigmentary traits (freckles, age spots, and tanning ability; Endo et al., 2018; Shido et al., 2019). For example, *BNC2* rs16935073 showed the most significant genetic effect on facial pigmented spots in this study. This variant was reported to be significantly associated with tanning ability in Shido et

al. (2019), and rs10816035 with age spots in Endo et al. (2018; pairwise r^2 between rs16935073 and rs10816035 = 0.91). Moreover, *MFSD12* rs2240751 showed the same direction of genetic effect on facial skin pigmentary traits in the Korean and Japanese populations. Of note, the *MFSD12* locus has been previously reported to be associated with skin pigmentation in African, and the association of *MFSD12* rs2240751 with skin pigmentary traits was also observed in the Latin American population (Adhikari et al., 2019; Crawford et al., 2017). The significant association of the chromosome chr10q26 genomic region led by rs11198112 was demonstrated by *HSPA12A* rs12259842 (imputed variant in this study with predicted allelic dosage *R*-squared value (DR2 imputation info score) of 0.97; *P*-value = 9.43×10^{-10}). This overall similarity in genetic effects on facial skin pigmentary traits may be explained by the shared genetic backgrounds of East Asian populations. Furthermore, associations between genetic variants of *BNC2*, *PPARGC1B*, *MFSD12*, and *MC1R* with skin pigmentary traits have been well reported in diverse populations (European, American, and African; Adhikari et al., 2019; Crawford et al., 2017; Endo et al., 2018; Eriksson et al., 2010; Jacobs et al., 2015; Liu et al., 2015; Shido et al., 2019; Visconti et al., 2018). *MC1R* is known to be a major factor influencing human skin pigmentation by regulating the type of melanin produced. Most GWAS analyses have reported that rs2228479 (p.Val92Met), rs1805007 (p.Arg151Gly), and rs1805008 (p.Arg160Trp) of *MC1R* strongly affected skin pigmentary traits (Liu et al., 2015; Peng et al., 2019; Stokowski et al., 2007). We could not test rs1805007 (p.Arg151Gly) or rs1805008 (p.Arg160Trp) in our GWAS analysis, since these SNPs were rare within this study's sample population (MAF < 0.5%). However, a nonsynonymous SNP (rs2228479, p.Val92Met) was polymorphic and showed an association signal with skin pigmentation related traits in East Asian populations in this study and a recent Japanese study (Endo et al., 2018), illustrating allelic heterogeneity in *MC1R*. Members of *PPARGC1* family

have been shown to be transcriptional coactivators for mitochondrial biogenesis regulation and other metabolic functions in several tissues (Handschin and Spiegelman., 2006; Rowe et al., 2010). *PPARGC1* genes act as a melanogenesis factor in melanocytes by activating *MITF* expression (Shoag et al., 2013). The association between skin pigmentation and genetic variants in *PPARGC1B* and *MITF*, which act as regulators of melanin synthesis in melanocytes, has been suggested in this study. Although the significance of *MITF* genetic variants did not reach the GWAS significance level in this study (data not shown), that of *PPARGC1B* did.

In addition, this study identified previously unreported loci to our knowledge near the long non-coding RNA (lncRNA) region associated with skin pigmentation. Non-coding RNAs such as microRNAs and lncRNAs have been shown to play a role in regulating post-transcriptional processes and epigenetic mechanisms (Jandura and Krause, 2017; Kopp and Mendell, 2018). Considering recent findings that the expression of microRNA and lncRNA in melanocytes changed by UV light stimulation and the expression of genes related to melanin synthesis was affected by microRNA and lncRNA levels, we speculated that variants near *lnc01877* and *CDKN2B-AS1* (long non-coding RNA located in the *CDKN2A-CDKN2B* gene clusters and influencing the expression of *CDKN2A* and *CDKN2B*, which play roles in cell cycle regulation) might affect biological processes, such as melanin synthesis, in melanocytes (Burd et al., 2010; Dynoodt et al., 2013, Kong et al., 2018, Zeng et al., 2016). As the underlying mechanism behind the association of lncRNA genetic variants and facial pigmented spots is unknown, further fine-mapping and validation need to be performed in independent studies on diverse populations.

In this study, for the fine-mapping of genomic regions that were significantly associated with facial pigmented spots (2q33, 5q32, 9p21, 9p22, 10q26, 16q24, and 19p13), *in*

silico based imputation analysis was additionally conducted. As a result of investigating genetic effects of the imputed variants on facial pigmented spots, the significance level of the imputed variants was found to be higher than that of the directly genotyped variants in three genomic regions (5q32, 9p21, and 16q24) for both the pigmented spots detected by UV light and polarized light (Supplementary Tables 1 and 2). Although previously unreported genomic regions associated with facial pigmented spots were not identified by imputation analysis, further studies through imputation based on larger population-specific reference panels may be valuable to identify novel loci, fine-map causal variants and examine functional annotations for facultative pigmentation.

Facial pigmented spots identified by different light sources have following characteristics: 1) when exposed to UV light (365 nm wavelength; UVA band), which can penetrate the dermis layer, melanin in the epidermal basal layer absorbs the light and the melanin-containing area is observed as black in the image (Brenner et al., 2008; Ou-Yang et al., 2004; Pérez-Sánchez et al., 2018), and 2) when exposed to polarized light, which can inhibit light reflectance from the epidermis, an area of the skin surface with different colors to adjacent regions is observed (Jacques et al., 2002). By using two types of light sources on pigmented spot identification (UV light and polarized light), we examined if genetic variations involved in pigmentation on the skin and pigmentation in the skin are different. However, the genetic variants associated with facial pigmented spots detected by the two light sources were overall the same. When quantifying the facial pigmented spots instead of body parts unexposed to UV, differences in the biological response (depending on the individual's genetic composition) to the environmental exposure (e.g., UV rays) can be observed.

This is the genome-wide investigation of genetic markers involved in facial pigmented spots in a Korean population. In this study, we demonstrated that genetic variants

of previously reported skin pigmentary trait genes (*BNC2*, *PPARGC1B*, *MC1R*, and *MFSD12*) showed significant effects on facultative skin pigmentation, through the initial GWAS and subsequent validation study. Moreover, to our knowledge, we have reported the association of SNPs near *lnc01877* and *CDKN2B-AS1* with facial pigmented spots at the genome-wide level. Further functional characterizations of the investigated genes are needed to fully evaluate their contribution to skin pigmentary traits.

MATERIALS & METHODS

Study subjects

In 2018, a total of 20,835 healthy Korean women were recruited as study subjects through cosmetic shops, which were subsidiaries of research institutions. All subjects agreed to participate in the following: 1) measurement of the degree of facial pigmented spots, 2) completion of a life-style questionnaire, and 3) collection of saliva for DNA testing. Facial pigmented spots were measured using the Janus 3 system. In detail, the Janus 3 system is a non-invasive method for evaluating facial skin characteristics by capturing an individual's entire facial image using three different light sources (normal light, UV light, and polarized light) with a 24.2-megapixel high resolution camera (Canon 200D DSLR, Sony, Japan). Images taken under UV and polarized light were used for analyzing facial pigmented spots (UV light captures the inside of epidermis and polarized light captures the skin surface). Captured image analysis was conducted with an internal algorithm which converted the images into numerical values (quantifying on a 100 percentage scale). For facial pigmented spots, the analysis detected areas that were hyper-pigmented (i.e. dark) relative to the surrounding skin, present on the forehead, nose, outer corner of the eyes, lower eyelids, and cheeks. Two sample images of the facial pigmented spots are shown in Supplementary Figure 7. The consistency of facial pigmented spot measurements using Janus 3 was confirmed by our previous study on repeated skin characteristics measurement studies using Janus 3 (Leem et al., 2020). Particularly, in three repeated measurements of a total of 70 subjects, the deviations of facial pigmented spots detected by both UV and polarized light in individuals were less than 1% in the quantification range of facial pigmented spots. Notably, to reduce intervening factors that could have affected the precision of the results, before capturing an

individual's facial image, all study subjects performed facial cleansing and waited for 30 minutes to equilibrate their skin status to normal condition. A blackout was used to block external light sources. Through the lifestyle questionnaire, data on age, sex, height, weight, average amount of sunlight exposure per day, sunscreen usage, average daily water intake, alcohol consumption, smoking frequency, average daily sleep duration, and eating habits were collected. Among the collected questionnaires, age was used as a covariate to adjust for potentially confounding factors for facial pigmented spots. For GWAS, the study subjects were divided into two groups, the discovery and the validation stage groups, according to the genotyping chip used. The discovery and validation stage groups consisted of 13,350 and 7,485 study samples, with an average age of 45.96 and 46.46 years, respectively. The institutional review board (IRB) at the LG H&H Research Center approved this study. All subjects were fully informed about the study and signed an IRB-approved written informed consent form.

Genome-wide SNP genotyping

A total of 13,350 DNA samples, from the discovery stage group, were genotyped using the Illumina Global Screening Array MD version 1 BeadChip (700,078 genetic markers, Illumina, San Diego, CA, USA), and 7,485 DNA samples, from the validation stage group, were genotyped using the Illumina Global Screening Array MD version 2 BeadChip (759,993 genetic markers, Illumina, San Diego, CA, USA). SNP genotyping was performed by Macrogen Inc. (Seoul, Korea). Samples were processed according to the recommendations of the Illumina Infinium HTS assay reference guide. Briefly, approximately 200 ng of genomic material was used to genotype each sample that had undergone whole-genome amplification, fragmentation, precipitation, and re-suspension in an appropriate hybridization buffer.

Denatured samples were hybridized on a prepared BeadChip for a minimum of 16 hours at 48 °C. Following hybridization, the BeadChips were processed for the single-base extension reaction, stained, and imaged using an Illumina iScan (BeadChip reading equipment). Normalized bead intensity data obtained for each sample were loaded into the GenomeStudio software (Illumina), which converted fluorescence intensity into SNP genotypes for each genetic marker.

QC of genotypic data for both the discovery and validation stages was performed. For sample QC, we excluded samples with a call rate lower than 98% or samples with a mismatch between self-reported and genetically inferred sex. Sex inference, based on genotype data, was performed by calculating the X chromosome heterozygosity rate. For variant QC, the following criteria were applied to remove inadequate results from the genotyping assay: (1) marker call rate < 98%, (2) number of alleles > 2, (3) minor allele frequency (MAF) < 1%, and (4) deviation from Hardy-Weinberg equilibrium (HWE *P*-value < 1×10^{-6}). Additionally, genetic markers with no chromosome information were also excluded. In order to reduce duplicated genetic effects and obtain reliable results, individuals with identity by descent greater than 0.25 (second degree) were excluded from subsequent GWAS analysis using KING (Manichaikul et al., 2010). After applying the QC criteria, 366,864 and 389,461 variants and 11,079 and 5,940 samples remained for the discovery and validation stages, respectively, which were used for further analysis. To examine possible stratification among our study populations, principal component analysis (PCA) of study subjects was also performed (Supplementary Figure 8). Data processing was performed using PLINK v1.9 (Chang et al., 2015).

Statistical analysis

Correlation analysis between facial pigmented spots and age, and BMI was performed using R version 3.5.1. For genome-wide association analysis, the statistical significance (*P*-value) of the associations and effect size (β-value) adjusted for age and 10 principal components as covariates, were assessed with linear regression for the additive model using the SVS HelixTree software (Golden Helix, Inc., Bozeman, Montana, USA). Bonferroni's correction for multiple testing was applied to the *P*-values based on the number of SNPs investigated. For validation, we selected variants that showed a significant association after Bonferroni's correction (*P*-value < 0.05/378,915). We also performed a meta-analysis of the discovery and validation samples for the significant variants using METAL (Willer et al., 2010). Regional plots of genomic regions around two previously unreported GWAS loci were obtained using the Locus zoom. LD was obtained using Haploview v4.2 software downloaded from the Broad Institute (http://www.broadinstitute.org/mpg/haploview), with examination of Lewontin's D′ (|*D′*|) and the LD coefficient r^2 between all pairs of biallelic loci (Barrett et al., 2015).

In order to achieve fine-mapping of the GWAS signal, genome-wide imputation analysis was conducted on discovery stage data using the program Beagle v 5.1 (Browning et al., 2007; Browning et al., 2018) with the 1000 Genomes Phase 3 reference panel (2,504 samples of admixed populations; 1000 Genomes Project Consortium et al., 2015). The parameters for imputation were set as the default values of the Beagle tool. After imputation analysis, 30,761,499 variants were obtained. The following criteria were applied as quality control for imputed variants: (1) imputation quality score (predicted allelic dosage *R*-squared value; DR2) < 0.3 (Gilly et al., 2019), (2) marker call rate < 98%, (3) MAF < 1%, and (4) deviation from HWE (HWE *P*-value < 1×10^{-6}), and the remaining 6,368,695 variants were used for subsequent analysis. For imputed variants, data processing and genome-wide

association analysis were performed using PLINK v1.9 (Chang et al., 2015) and SVS HelixTree software (Golden Helix, Inc., Bozeman, Montana, USA).

DATA AVAILABILITY STATEMENT

Raw genotype or phenotype data cannot be made available due to restrictions imposed by the ethics approval. Summary statistics for this study is deposited at NHGRI-EBI GWAS catalog with link.

1) facial pigmented spots detected by ultraviolet light (study accession no. GCST90002283) ftp://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90002283;

2) facial pigmented spots detected by polarized light (study accession no. GCST90002284) ftp://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90002284

ABBREVIATIONS

GWAS, genome-wide association study; lncRNA, long non-coding RNA; MAF, minor allele frequency; QC, quality control; Q-Q plot, quantile-quantile plot; SNP, single nucleotide polymorphism; UV, ultraviolet

CONFLICT OF INTEREST

All authors claim no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: JGS, SL, HHW, NGK; Methodology: JGS, SL, BK; Formal Analysis: JGS, SL; Investigation: JGS, SL, YK; Resources: YK, SGP, NGK; Data Curation: JGS, YK, HJS, SGL, JYS; Writing - Original Draft Preparation: JGS; Writing - Review and Editing: JGS, SL, YK, HHW, NGK; Visualization: JGS, BK; Supervision: HHW, NGK; Funding Acquisition: NGK

REFERENCES

1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, et al. **A global reference for human genetic variation.** Nature. 2015; 526: 68-74.

Adhikari K, Mendoza-Revilla J, Sohail A, Fuentes-Guajardo M, Lampert J, Chacon-Duque JC, et al. **A GWAS in Latin Americans highlights the convergent evolution of lighter skin pigmentation in Eurasia.** Nat Commun. 2019; 10: 1-16.

Barrett JC, Fry B, Maller J, Daly MJ. **Haploview: analysis and visualization of LD and haplotype maps.** Bioinformatics. 2005; 21: 263-265.

Beleza S, Johnson NA, Candille SI, Absher DM, Coram MA, Lopes J, et al. **Genetic architecture of skin and eye color in an African-European admixed population.** PLoS Genet. 2013; 9: e1003372.

Brenner M, Hearing VJ. **The protective role of melanin against UV damage in human skin.** Photochem Photobiol. 2008; 84: 539-549.

Browning BL, Zhou Y, Browning SR. **A One-Penny Imputed Genome from Next-Generation Reference Panels.** Am J Hum Genet. 2018; 103: 338-348.

Browning SR, Browning BL. **Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering.** Am J Hum Genet. 2007; 81: 1084-1097.

Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z, Sharpless NE. **Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk.** PLoS Genet. 2010; 6: e1001233.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. **Second-generation PLINK: rising to the challenge of larger and richer datasets.** GigaScience. 2015; 4: 7. s13742-015.

Crawford NG, Kelly DE, Hansen MEB, Beltrame MH, Fan S, Bowman SL, et al. **Loci associated with skin pigmentation identified in African populations.** Science. 2017; 358: eaan8433.

Dąbrowska AK, Spano F, Derler S, Adlhart C, Spencer ND, Rossi RM. **The relationship between skin function, barrier properties, and body-dependent factors.** Skin Res Technol. 2018; 24: 165-174.

Del Bino S, Duval C, Bernerd F. **Clinical and Biological Characterization of Skin Pigmentation Diversity and Its Consequences on UV Impact.** Int J Mol Sci. 2018; 19: 2668.

Dynoodt P, Mestdagh P, Van Peer G, Vandesompele J, Goossens K, Peelman LJ, et al. **Identification of miR-145 as a key regulator of the pigmentary process.** J Invest Dermatol. 2013; 133: 201-209.

Endo C, Johnson TA, Morino R, Nakazono K, Kamitsuji S, Akita M, et al. **Genome-wide association study in Japanese females identifies fifteen novel skin-related trait associations.** Sci Rep. 2018; 8: 1-22.

Eriksson N, Macpherson JM, Tung JY, Hon LS, Naughton B, Saxonov S, et al. **Web-based, participant-driven studies yield novel genetic associations for common traits.** PLoS Genet. 2010; 6: e1000993.

Foo YZ, Simmons LW, Rhodes G**. Predictors of facial attractiveness and health in humans.** Sci Rep. 2017; 7: 39731.

Gilly A, Southam L, Suveges D, Kuchenbaecker K, Moore R, Melloni GEM, et al. **Very low-depth whole-genome sequencing in complex trait association studies.** Bioinformatics. 2019; 35: 2555-2561.

Goo BL, Kang JS, Cho SB. **Treatment of early-stage erythematotelangiectatic rosacea with a Q-switched 595-nm Nd:YAG laser.** J Cosmet Laser Ther. 2015; 17: 139-142.

Handschin C, Spiegelman BM. **Peroxisome proliferator-activated receptor γ coactivator 1 coactivators, energy homeostasis, and metabolism.** Endocr Rev. 2006; 27: 728-735.

Hillebrand GG, Miyamoto K, Schnell B, Ichihashi M, Shinkura R, Akiba S, et al. **Quantitative evaluation of skin condition in an epidemiological survey of females living in northern versus southern Japan.** J Dermatol Sci. 2001; 27: 42-52.

Jacobs LC, Wollstein A, Lao O, Hofman A, Klaver CC, Uitterlinden AG, et al. **Comprehensive candidate gene study highlights UGT1A and BNC2 as new genes determining continuous skin color variation in Europeans.** Hum Genet. 2013; 132: 147-158.

Jacobs LC, Hamer MA, Gunn DA, Deelan J, Lall JS, van Heemst D, et al. **A genome-wide association study identifies the skin color genes IRF4, MC1R, ASIP, and BNC2 influencing facial pigmented spots.** J Invest Dermatol. 2015; 135: 1735-1742.

Jacques SL, Ramella-Roman JC, Lee K. **Imaging skin pathology with polarized light.** J Biomed Opt. 2002; 7: 329-340.

Jandura A, Krause HM. **The new RNA world: growing evidence for long noncoding RNA functionality.** Trends Genet. 2017; 33: 665-676.

Kim HK, Min KO, Choi JH, Kim SH. **Effects of low-level laser therapy, electroacupuncture, and radiofrequency on the pigmentation and skin tone of adult women.** J Phys Ther Sci. 2016; 28: 1407-1411.

Kong Y, Hsieh CH, Alonso LC. **ANRIL: A lncRNA at the CDKN2A/B Locus With Roles in Cancer and Metabolic Disease.** Front Endocrinol (Lausanne). 2018; 24: 405.

Kopp F, Mendell JT. **Functional classification and experimental dissection of long noncoding RNAs.** Cell. 2018; 172: 393-407.

Lee MH, Lee KK, Park MH, Hyun SS, Kahn SY, Joo KS, et al. **In vivo anti-melanogenesis activity and in vitro skin permeability of niacinamide-loaded flexible liposomes (Bounsphere™).** J Drug Deliv Sci Technol. 2016; 31: 147-152.

Leem S, Chang J, Kim Y, Shin JG, Song HJ, Lee SG, et al. **Repeated measurements of facial skin characteristics using the Janus-Ⅲ measurement system.** Skin Res Technol. 2020; 26: 362-368.

Liu F, Visser M, Duffy DL, Hysi PG, Jacobs LC, Lao O, et al. **Genetics of skin color variation in Europeans: genome-wide association studies with functional follow-up.** Hum Genet. 2015; 134: 823-835.

Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. **Robust relationship inference in genome-wide association studies. Bioinformatics.** 2010; 26: 2867-2873.

McCarroll SA, Hyman SE. **Progress in the genetics of polygenic brain disorders: significant new challenges for neurobiology.** Neuron. 2013; 80: 578-587.

Nan H, Kraft P, Hunter DJ, Han J. **Genetic variants in pigmentation genes, pigmentary phenotypes, and risk of skin cancer in Caucasians.** Int J Cancer. 2009; 125: 909-917.

Ou-Yang H, Stamatas G, Kollias N. **Spectral responses of melanin to ultraviolet A irradiation.** J Invest Dermatol. 2004; 122: 492-496.

Parra EJ. **Human pigmentation variation: evolution, genetic basis, and implications for public health.** Am J Phys Anthropol. 2007; 134: 85-105.

Pavan WJ, Sturm RA. **The genetics of human skin and hair pigmentation. Annu Rev Genomics Hum Genet.** 2019; 20: 41-72.

Peng F, Zhu G, Hysi PG, Eller RJ, Chen Y, Li Y, et al. **Genome-wide association studies identify multiple genetic loci influencing eyebrow color variation in Europeans.** J Invest Dermatol. 2019; 139: 1601-1605.

Pérez-Sánchez A, Barrajón-Catalán E, Herranz-López M, Micol V. **Nutraceuticals for Skin Care: A Comprehensive Review of Human Clinical Studies.** Nutrients. 2018; 10: 403.

Pezic A, Ponsonby AL, Cameron FJ, Rodda C, Ellis JA, Halliday J, et al. **Constitutive and relative facultative skin pigmentation among Victorian children including comparison of two visual skin charts for determining constitutive melanin density.** Photochem Photobiol. 2013; 89: 714-723.

Praetorius C, Grill C, Stacey SN, Metcalf AM, Gorkin DU, Robinson KC, et al. **A polymorphism in IRF4 affects human pigmentation through a tyrosinase-dependent MITF/TFAP2A pathway**. Cell. 2013; 155: 1022-1033.

Rowe GC, Jiang A, Arany Z. **PGC-1 coactivators in cardiac development and disease.** Circ Res. 2010; 107: 825-838.

Shekar SN, Luciano M, Duffy DL, Martin NG. **Genetic and environmental influences on skin pattern deterioration.** J Invest Dermatol. 2005; 125: 1119-1129.

Shido K, Kojima K, Yamasaki K, Hozawa A, Tamiya G, Ogishima S, et al. **Susceptibility Loci for Tanning Ability in the Japanese Population Identified by a Genome-Wide Association Study from the Tohoku Medical Megabank Project Cohort Study.** J Invest Dermatol. 2019; 139: 1605-1608.

Shoag J, Haq R, Zhang M, Liu L, Rowe GC, Jiang A, et al. **PGC-1 coactivators regulate MITF and the tanning response.** Mol Cell. 2013; 49: 145-157.

Sim JH, Park YL, Lee JS, Lee SY, Choi WB, Kim HJ, et al. **Treatment of melasma by low-fluence 1064 nm Q-switched Nd:YAG laser.** J Dermatolog Treat. 2014; 25: 212-217.

Stokowski RP, Pant PV, Dadd T, Fereday A, Hinds DA, Jarman C, et al. **A genomewide association study of skin pigmentation in a South Asian population.** Am J Hum Genet. 2007; 81: 1119-1132.

Visconti A, Duffy DL, Liu F, Zhu G, Wu W, Chen Y, et al., **Genome-wide association study in 176,678 Europeans reveals genetic loci for tanning response to sun exposure.** Nat Commun. 2018; 9: 1-7.

Willer CJ, Li Y, Abecasis GR. **METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics.** 2010; 26: 2190-2191.

Zeng Q, Wang Q, Chen X, Xia K, Tang J, Zhou X, et al. **Analysis of lncRNAs expression in UVB-induced stress responses of melanocytes.** J Dermatol Sci. 2016; 81: 53-60.

Table 1. Characteristics of study subjects

Characteristics	Total (n = 17,019)	Discovery stage (n = 11,079)	Validation stage (n = 5,940)
Age (years)	46.13 ± 11.47	45.96 ± 11.09	46.46 ± 12.13
Facial pigmented spots			
UV light (percentage)	35.55 ± 12.26	35.45 ± 12.15	35.74 ± 12.45
Polarized light (percentage)	27.33 ± 10.08	27.26 ± 9.94	27.47 ± 10.33

All values are mean ± standard deviation
Abbreviation: UV, ultraviolet

Table 2. Summary statistics of GWAS: Significantly associated genetic variants in the genome-wide association analysis of facial pigmented spots

Trait	SNP	Gene	Position	Genomic region	Previous study		GWAS stage			Validation stage			Meta-analysis	
					Reported trait	Population	MAF	*P*-value	β[1]	MAF	*P*-value	β[1]	MAF	*P*-value
Facial pigmented spots detected by UV light	rs16935073	*BNC2*	intron	9p22	Tanning response	Japanese	0.434	4.02E-35	1.58	0.434	3.80E-15	1.34	0.434	2.11E-46
	rs32579	*PPARGC1B*	intron	5q32	Tanning ability	European	0.295	3.59E-29	-1.56	0.302	2.06E-15	-1.43	0.297	9.04E-42
	rs11198112	-	intergenic	10q26	Skin pigmentation[4]	Latin american	0.097	1.17E-26	-2.30	0.097	7.85E-14	-2.08	0.097	9.66E-38
	rs2228479	*MC1R*	exon (Val92Met)	16q24	Skin color	European	0.136	3.03E-17	1.55	0.134	1.44E-06	1.16	0.135	6.62E-21
	rs12693889[2]	*lnc01877*	intron	2q33	-		0.500	3.91E-09	0.75	0.497	2.69E-04	0.60	0.499	1.59E-11
	rs643319[2,3]	*CDKN2B-AS1*	intron	9p21	-		0.363	3.76E-07	-0.67	0.366	2.00E-03	-0.54	0.364	7.76E-09
	rs2240751[3]	*MFSD12*	exon (U182H)	19p13	Tanning response	Japanese	0.335	7.27E-07	-0.67	0.337	1.59E-03	-0.56	0.336	9.70E-09
Facial pigmented spots detected by polar light	rs16935073	*BNC2*	intron	9p22	Tanning response	Japanese	0.434	9.62E-23	0.99	0.434	1.24E-11	0.93	0.434	8.75E-32
	rs32579	*PPARGC1B*	intron	5q32	Tanning ability	European	0.295	4.19E-22	-1.06	0.302	1.41E-11	-0.98	0.297	3.54E-31
	rs11198112	-	intergenic	10q26	Skin pigmentation[4]	Latin american	0.097	1.07E-18	-1.49	0.097	1.54E-09	-1.35	0.097	7.52E-26
	rs2240751	*MFSD12*	exon (U182H)	19p13	Tanning response	Japanese	0.335	2.12E-10	-0.67	0.337	8.47E-06	-0.63	0.336	2.16E-14
	rs2228479	*MC1R*	exon (Val92Met)	16q24	Skin color	European	0.136	1.74E-10	0.92	0.134	6.40E-04	0.66	0.135	4.29E-12
	rs643319[2]	*CDKN2B-AS1*	intron	9p21	-		0.363	4.50E-08	-0.57	0.366	2.12E-04	-0.52	0.364	8.75E-11
	rs12693889[2]	*lnc01877*	intron	2q33	-		0.500	2.42E-08	0.56	0.497	2.14E-03	0.41	0.499	9.61E-10

1, effect size of minor allele (see Supplementary Tables 1 and 2 for major and minor alleles)

2, a previously unreported association with facial pigmented spots to our knowledge.

3, rs643319 and rs2240751 were marginally associated with facial pigmented spots detected by UV light in the discovery stage sample.

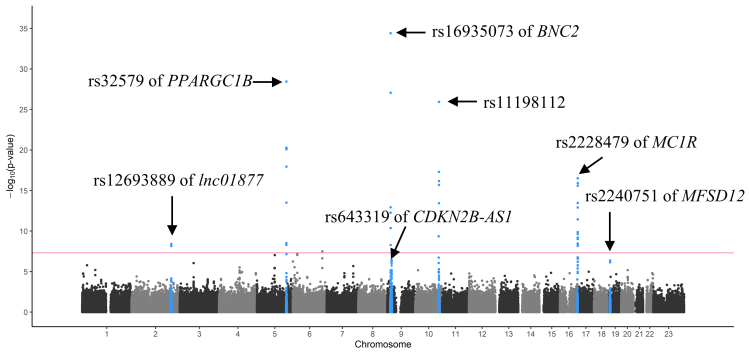
4, constitutive skin pigmentation

Abbreviations: GWAS, genome-wide association study; SNP, single nucleotide polymorphism; MAF, minor allele frequency; UV, ultraviolet

FIGURE LEGENDS

Figure 1. **Manhattan plot for genome-wide association analysis of facial pigmented spots in the discovery stage.** Manhattan plots with $-\log_{10}$(*P*-value) are presented for two facial pigmented spots detected by (a) UV light and (b) polarized light. The horizontal line indicates the GWAS significance (red line corresponds to genome-wide significance threshold after Bonferroni's correction, *P*-value = 1.32×10^{-7}) threshold that was used for identifying association signals. Arrow indicates the lead SNPs in the associated genomic region. Chromosome X was designated as 23 on X-axis.

a.



b.