GWAS Analysis of 17,019 Korean Women Identifies the Variants Associated with Facial Pigmented Spots

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Variation in skin pigmentation can be affected by both environmental factors and intrinsic factors such as age, gender, and genetic variation. Recent GWASs revealed that genetic variants of genes functionally related to a pigmentation pathway were associated with skin pigmentation traits. However, these GWASs 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 pigmentation 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 × 10⁻⁴⁸), MC1R at 16q24 (rs2228479; P-value = 6.62 × 10⁻⁴¹), Inc01877 at 2q33 (rs12693889; P-value = 1.59 × 10⁻¹²), CDKN2B-AS1 at 9p21 (rs643319; P-value = 7.76 × 10⁻⁸), 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.

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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 the perception of different sensations, and regulating body temperature (Dabrowska 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 pigmentation 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 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 pigmentation 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 pigmentation traits, several common genetic variants act in a probabilistic rather than deterministic fashion. GWAS, 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 pigmentation 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 the 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 (Illumina, San Diego, CA) to identify the 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, 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 S1a. The facial pigmented spots of study subjects, detected by UV light, were similar between 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. The facial pigmented spots detected by both UV light and polarized light were highly correlated (Supplementary Figure S1b). The amount of skin pigmentation increased with age similarly in both the discovery and the validation stages, with an average value of 35.45 and 35.74, 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 Inc01877 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 the intronic regions of Inc01877 and CDKN2B-AS1, respectively. Regional plot and linkage disequilibrium pattern of genomic regions for the two loci are presented in Supplementary Figures S5 and S6.

GWAS analysis of facial pigmented spots

In the discovery stage, a total of 13,350 Korean women were genotyped using an SNP microarray chip. After applying the quality control (QC) criteria of genotype data, 366,864 genetic markers from 11,079 samples were used for GWAS analysis. A quantile–quantile plot was used to test the validity of the distributional assumption for the GWAS datasets. The quantile–quantile 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 S4a and b).

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 and Supplementary Tables S1 and S2).

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 quantile–quantile plot was used to test the validity of the distributional assumption for genome-wide level testing (Supplementary Figure S4c and d). The genetic effects of lead SNPs of the 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 and rs2228479 (p.Val92Met) of MCIR, 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 Inc01877 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 the intronic regions of Inc01877 and CDKN2B-AS1, respectively. Regional plot and linkage disequilibrium pattern of genomic regions for the two loci are presented in Supplementary Figures S5 and S6.

DISCUSSION

Skin pigmentation induced by extrinsic factors such as exposure to UV rays and nonuse of sunscreen is known as facultative skin pigmentation, whereas 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). 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 the skin pigmentary traits, studies in diverse populations are still needed. To investigate the 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 (McCarroll 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.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (N = 17,019)</th>
<th>Discovery Stage (n = 11,079)</th>
<th>Validation Stage (n = 5,940)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.13 ± 11.47</td>
<td>45.96 ± 11.09</td>
<td>46.46 ± 12.13</td>
</tr>
<tr>
<td>Facial pigmented spots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV light (%)</td>
<td>35.55 ± 12.26</td>
<td>35.45 ± 12.15</td>
<td>35.74 ± 12.45</td>
</tr>
<tr>
<td>Polarized light (%)</td>
<td>27.33 ± 10.08</td>
<td>27.26 ± 9.94</td>
<td>27.47 ± 10.33</td>
</tr>
<tr>
<td>All values are mean ± SD.</td>
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</tbody>
</table>

Table 1. Characteristics of Study Subjects
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 this 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 was reported to be significantly associated 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 the African population, 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

![Manhattan plots](image)

**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 the 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.
### Table 2. Summary Statistics of GWAS: Significantly Associated Genetic Variants in the GWAS analysis of Facial Pigmented Spots

<table>
<thead>
<tr>
<th>Trait</th>
<th>SNP</th>
<th>Gene</th>
<th>Position</th>
<th>Genomic Region</th>
<th>Reported Trait</th>
<th>Population</th>
<th>MAF</th>
<th>P-value</th>
<th>β†</th>
<th>MAF</th>
<th>P-value</th>
<th>β†</th>
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<td></td>
</tr>
<tr>
<td>detected by UV light</td>
<td>rs16935073</td>
<td>BNC2</td>
<td>Intron</td>
<td>9p22</td>
<td>Tanning response</td>
<td>Japanese</td>
<td>0.434</td>
<td>4.02E-35</td>
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<td>Tanning ability</td>
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<td>3.59E-29</td>
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<td>0.302</td>
<td>2.06E-15</td>
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<td>0.297</td>
<td>9.04E-42</td>
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<td>Skin pigmentation</td>
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<td>1.17E-26</td>
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<td>3.03E-17</td>
<td>1.55</td>
<td>0.134</td>
<td>1.44E-06</td>
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<td>0.135</td>
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<td>Intron</td>
<td>2q33</td>
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<td>3.91E-09</td>
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<td>0.497</td>
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<td>0.60</td>
<td>0.499</td>
<td>1.59E-11</td>
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<td></td>
<td>0.363</td>
<td>3.76E-07</td>
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<td>0.366</td>
<td>2.00E-03</td>
<td>-0.54</td>
<td>0.364</td>
<td>7.76E-09</td>
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<td><strong>Facial pigmented spots</strong></td>
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<td>detected by polar light</td>
<td>rs2240751</td>
<td>MFSD12</td>
<td>Exon (U182H)</td>
<td>19p13</td>
<td>Tanning response</td>
<td>Japanese</td>
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<td>Intron</td>
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<td>2.42E-08</td>
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<td>2.14E-03</td>
<td>0.41</td>
<td>0.499</td>
<td>9.61E-10</td>
</tr>
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</table>

Abbreviation: MAF, minor allele frequency.

†Effect size of the minor allele (see Supplementary Tables S1 and S2 for major and minor alleles).

2Constitutive skin pigmentation.

3A previously unreported association with facial pigmented spots, to our knowledge.

4rs643319 and rs2240751 were marginally associated with facial pigmented spots detected by UV light in the discovery stage sample.
the chromosome chr10q26 genomic region led by rs11198112 was demonstrated by HSPA12A rs12259842 (the imputed variant in this study with predicted allelic dosage $R^2$ value [imputation info score] of 0.97; $P$-value = $9.43 \times 10^{-10}$). This overall similarity in the genetic effects on facial skin pigmentation traits may be explained by the shared genetic backgrounds of East Asian populations. Furthermore, associations between genetic variants of BNC2, PPARGC1B, MFSM12, and MC1R with skin pigmentation 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 melatin produced. Most GWAS analyses have reported that rs2228479 (p.Val92Met), rs1805007 (p.Arg151Gly), and rs1805008 (p.Arg160Trp) of MC1R strongly affected skin pigmentation 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 because these SNPs were rare within this study’s sample population (minor allele frequency < 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 the 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 noncoding RNA (lncRNA) region associated with skin pigmentation. Noncoding RNAs such as microRNAs and IncRNAs have been shown to play a role in regulating post-transcriptional processes and epigenetic mechanisms (Jandura and Krause, 2017; Kopp and Mendell, 2018). Considering the recent findings that the expression of microRNA and IncRNA in melanocytes changed by UV light stimulation and the expression of genes related to melanin synthesis was affected by microRNA and IncRNA levels, we speculated that the variants near Inc01877 and CDKN2B-AS1 (lncRNA 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; Dynooldt et al., 2013; Kong et al., 2018; Zeng et al., 2016). Because the underlying mechanism behind the association of IncRNA 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 the 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 the pigmented spots detected by both UV light and polarized light (Supplementary Tables S1 and S2). 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 the following characteristics: (i) 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 and Hearing, 2008; Ou-Yang et al., 2004; Pérez-Sánchez et al., 2018), and (ii) 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 the two types of light sources on pigmented spot identification (UV light and polarized light), we examined whether the 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 the 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 pigmentation trait genes (BNC2, PPARGC1B, MC1R, and MFSM12) 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 Inc01877 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 AND 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: (i) measurement of the degree of facial pigmented spots, (ii) completion of a lifestyle questionnaire, and (iii) collection of saliva for DNA testing. Facial pigmented spots were measured using the Janus 3 system. In detail, the Janus 3 system
is a noninvasive 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, Tokyo, 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 hyperpigmented (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 S7. 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 <1% in the quantification range of facial pigmented spots. Notably, to reduce the 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, the 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, respectively, with an average age of 45.96 and 46.46 years, respectively. The institutional review board at the LG Household & Healthcare Research Center (Seoul, South Korea) approved this study. All subjects were fully informed about the study and signed an institutional review board–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), 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). SNP genotyping was performed by Macrogen (Seoul, Korea). Samples were processed according to the instructions of the Illumina Infinium HTS assay reference guide (Illumina). Briefly, approximately 200 ng of genomic material was used to genotype each sample that had undergone whole-genome amplification, fragmentation, precipitation, and resuspension in an appropriate hybridization buffer. Denatured samples were hybridized on a prepared BeadChip for a minimum of 16 hours at 48 °C. After hybridization, the BeadChips were processed for the single-base extension reaction, stained, and imaged using an Illumina iScan (BeadChip reading equipment; Illumina). 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 <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: (i) marker call rate of <98%, (ii) number of alleles of >2, (iii) minor allele frequency of <1%, and (iv) deviation from Hardy–Weinberg equilibrium (P-value < 1 × 10⁻⁵). In addition, the genetic markers with no chromosome information were also excluded. To reduce duplicated genetic effects and obtain reliable results, individuals with identity by descent of >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 of study subjects was also performed (Supplementary Figure S8). Data processing was performed using PLINK, version 1.9 (Chang et al., 2015).

**Statistical analysis**

Correlation analysis between facial pigmented spots and age and body mass index was performed using R, version 3.5.1 (R Core Team, 2014). For GWAS 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 SNP & Variation Suite HelixTree software (Golden Helix, Bozeman, Montana). Bonferroni’s correction for multiple testing was applied to the P-values on the basis of the number of SNPs investigated. For validation, we selected the variants that showed a significant association after Bonferroni’s correction (P-value < 0.05 per 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 LocusZoom (Prum et al., 2010). Linkage disequilibrium was obtained using Haploview, version 4.2, software downloaded from the Broad Institute (http://www.broadinstitute.org/mpg/haploview), with an examination of Lewontin’s D² (|D²|) and the linkage disequilibrium coefficient R² between all pairs of biallelic loci (Barrett et al., 2005).

To achieve fine mapping of the GWAS signal, genome-wide imputation analysis was conducted on the discovery stage data using the program Beagle, version 5.1 (Browning and Browning, 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 QC for imputed variants: (i) imputation quality score (predicted allelic dosage R² value) of <0.3 (Gilly et al., 2019), (ii) marker call rate of <98%, (iii) minor allele frequency of <1%, and (iv) deviation from Hardy–Weinberg equilibrium (P-value < 0.05 per 378,915).
1×10^{-8}), 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, version 1.9 (Chang et al., 2015), and SNP & Variation Suite HelixTree software.

Data availability statement
Raw genotype or phenotype data cannot be made available owing to restrictions imposed by the ethics approval. Summary statistics for this study are deposited at the NHGRI-EBI GWAS catalog with the following link:

1. facial pigmented spots detected by UV light (study accession number GCST90002283) (ftp://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90002283);

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CONFLICT OF INTEREST
The authors state no conflicts of interest.

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AUTHOR CONTRIBUTIONS
Conceptualization: J-G Shin, SL, HHW, NGK; Methodology: JG, SL, BK; Resources: YK, SGP, NGK; Supervision: JYS; Formal Analysis: JG, SL; Funding Acquisition: NGK; Investigation: JG, YK, HJS, SGL; Data Curation: JG, YK, HJS, SGL.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.08.007.

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