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Identification of the Underlying Genetic Factors of Skin Aging in a Korean Population Study

JUNG OH KIM, BOREUM PARK, JOUNG YOON CHOI, SO RA LEE, SO JIN YU, MYEONGJIN GOH, HAEKWANG LEE, WON-SEOK PARK, IN SOO SUH, DAE-SEUNG KOH, and KYUNG-WON HONG, *Theragen Etex Bio Institute Co. Ltd., Suwon 16229, Republic of Korea (J.O.K., B.P., J.Y.C., S.R.L., S.J.Y., K.W.H.), Amorepacific R&D Center, Amorepacific Corporation, Yongin 17074, Republic of Korea (M.G.), P&K Skin Research Center, Yeongdeungpo-gu, Seoul 07236, Republic of Korea (H.L., W.-S.P.), Bio-Convergence Center, Jeju Technopark, Jeju 63243, Republic of Korea (I.S.S., D.S.K.)*

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Synopsis

Genetic polymorphisms may affect the molecular mechanisms underlying determination of skin type. So far, several genetic studies have been reported; however, very few studies have been conducted to examine the relationship between genotype and skin phenotypes. In this study, the genome sequences of individuals tested for five cosmetic characteristics (wrinkles, moisture content, pigmentation, oil content, and sensitivity) were determined, and we also conducted five genome-wide association studies (GWASs) to identify predictive markers. Some single-nucleotide polymorphisms (SNPs) within those genes were more frequent in individuals exhibiting stronger traits. GWASs revealed that two genome-wide significant SNPs within *FCRL5* and *OCA2* genes were associated with wrinkles and pigmentation, respectively ($p < 5 \times 10^{-8}$), and that genome-wide SNPs in 21 genes (wrinkles: *FCRL5*, *REEP3*, *ADSS*, and *SPTLC1*; moisture: *TBX4*, *TRPM3*, *CEMIP2*, *CTSH*, and *TTC39C*; pigmentation: *OCA2*, *NCLN*, *TNS1*, *CDC42BPA*, *HS3ST4*, and *UNCX*; oil: *SYN2*, *CNDP1*, *GAS6*, *INSR*, and *TNFRSF19*; and sensitivity: *CREB5*) might be associated with five skin phenotypes. Among these, a genome-wide significant SNP (rs117381658) and the SNP located downstream of *FCRL5*, which encodes a member of the immunoglobulin receptor family, were associated with an increased risk of wrinkles ($p = 1.52 \times 10^{-8}$). The other genome-wide significant SNP (rs74653330) was associated with a decreased risk of pigmentation ($p = 1.04 \times 10^{-8}$), which is located in the coding region of *OCA2* that encodes for a transporter of melanin. Our study reports genetic factors associated with skin cosmetology parameters in the Korean population. We hope our findings will provide a foundation for further genetic and molecular studies related to custom cosmetics. Based on these findings, the industry will be able to provide consumers with ingredients capable of palliating the lack of function associated in genes with SNPs.

INTRODUCTION

Skin aging is recognized as a clinically observable alteration of mechanical and chromatic properties on the skin's surface. In cosmetic dermatology, wrinkling is an obvious and

Address all correspondence to Kyung-Won Hong at kyungwon.hong@theragenetex.com.

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very important indicator of aging. In addition, all skin types can develop hyperpigmentary problems with aging, for example, postinflammatory hyperpigmentation, solar lentigos, and melanoma (1). The importance of skin aging tends to vary in degree according to age, gender, individual lifestyle, and inherited genetic susceptibility (2). Although external factors are known to be important risk factors in skin changes, they do not explain individual differences in the degree of these changes, despite many previous studies on this topic. Interestingly, the differences in the degree of change for each skin indicator reflect individual characteristics that are independent of external factors. In addition, the interest in skin indicators may vary according to age-group and individual skin conditions (3). In particular, individuals with skin-related diseases or problems are likely to be interested in relevant skin indicators. Therefore, studies (2–11) are being actively conducted to understand the differences in individual skin changes, such as aging, dryness, freckles, wrinkles, and sensitivity.

Individual skin characteristics are mostly congenital features inherited from one's parents, and changes in response to external factors are often attributed to these characteristics. Therefore, it is essential to study genes to understand the differences in the degree of change in skin indicators, and previous studies have reported some skin-related gene variants. These skin-related gene variants have been shown to correlate with an increased risk of developing certain diseases, such as erythema and skin cancer (*MYH7B* and *PIGU*, respectively), and with cosmetic appearance-related traits, such as sun sensitivity (*ASIP*) (12), hydration (*SSBP*), pigmentation (*OCA2*, *TPCN2*, and *ASIP*) (12,13), wrinkles (*SHC4* and genes on 10q26.13) (14), freckles (*TYR*, *OCA2*, and *MC1R*) (13), and visible aging (*DIAPH2* and *EDEM1*) (15). There are also many reports of genetic variation related to skin characteristics.

Furthermore, previous reports have indicated that the expression of several genes correlates with changes in skin conditions by using various strategies, including functional studies, animal experiments, and large-scale population studies. Changes in *ASIP* expression are associated with depigmentation of the epidermis in rats (16), and the *OCA2* single-nucleotide polymorphism (SNP), rs1800414 (His1844Arg), was identified in a skin pigmentation reaction study, in which 1,159 individuals living in southwestern China and 359 people living in Cambodia were included (17). Regarding the functional studies, the amount of melanocytes decreased when *OCA2* knockout was performed in zebra fish and mouse experiments (17). *TPCN2* affects the size and pH control of melanosomes in human melanocytes (18), and *MC1R* was tested in 13,017 middle-aged Caucasian women in France to assess their sensitivity to the sun (19), skin pigmentation, and freckles. In this study, 15 genetic variants were identified (Arg151Cys, Arg160Trp, Arg142His, Asp294His, Ile155Thr, Asp84Glu, Val60Leu, Val92Met, Arg163Gln, Ser83Pro, Thr95Met, Pro256Ser, Val265Ile, Ala166Ala, and Gln233Gln) and analyzed to determine its correlation with skin features (19). Through this, it was found that *MC1R* polymorphisms may be associated with sensitivity to ultraviolet-induced DNA damage.

Moreover, a number of genome-wide association studies (GWASs) have been published, and many disease-associated genetic indices have been identified (20). According to the recently reported European GWAS results (21), 42 studies on human phenotypes have been identified and multiple loci associated with skin-related properties such as male baldness, unibrow, jaw dimple, and nose size have been reported. However, most large-scale association studies were performed in population samples that were predominantly of European ancestry. Furthermore, many studies evaluating specific traits present in

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various populations are actively being conducted, and data on new genetic variations and characteristics by race have been reported (22–24). However, the available skin GWAS results are largely limited to the traits of skin pigmentation, sensitivity to the sun, and inflammation. Although GWASs of pigmentation and sensitivity have been published, no such studies exist regarding the Korean population. The aim of this study was to identify the indicators of skin changes based on genetic variants specific to Koreans and to provide personalized solutions based on these indicators.

MATERIALS AND METHODS

PARTICIPANTS

The sample population investigated in this study consisted of 1,079 Korean women recruited between January 2019 and November 2019 at P&K Skin Research Center (Seoul, Korea). All recruited participants were female without skin-related diseases, and their average age was 40.81 years (Table I). All participants provided written informed consent, and this study was approved by the Institutional Review Board of the Theragen Etex Bio Institute (IRB No.: 700062-20190819-GP-006-01).

MEASUREMENT OF SKIN PHENOTYPES

To measure skin traits, various measuring devices were used. For wrinkles, a Primos CR (Canfield Scientific, Parsippany, NJ) was used to perform measurements at wrinkle sites, including the average roughness on the side of the eye, the maximum wrinkle depth on the side of the eye, the average roughness of the glabella, and the maximum wrinkle depth of the glabella. A CM-825 Corneometer® (EnviroDerm Services Ltd., Hedworth, Grange Court, United Kingdom.) was used to measure moisture content of the glabella and cheek. Pigmentation was divided into melanin and brightness categories using a Mexameter® MX 18 (Courage + Khazaka electronic GmbH, Köln, Germany) and a CM-700d (Konica Minolta inc., Tokyo, Japan) instrument, respectively. To identify the skin oil content, the average oil content was measured using an SM 815 Sebumeter® (Courage + Khazaka electronic GmbH., Köln, Germany) on the glabella and right cheek. Finally, we performed a sensitivity assay in which a 10% lactic acid treatment was applied to the skin over time; no measuring device was used for this assay because skin sensitivity is specific to the individual, and individual sensitivity was identified on the basis of the skin's reaction.

TARGET SKIN PHENOTYPE GRADING SCALE

It can be complicated to use measured values to perform GWASs because the measured values for the same phenotype may differ according to the measurement instrument. To solve this problem, we scored each measurement item using codes. The measured values for each item were divided into tertile criteria to delineate groups, and scoring was performed for each tertile group to quantify the integrated score for the target phenotype (Table II). Therefore, we summarized the total score for the "target phenotype for GWAS" and analyzed each phenotype.

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Table I
Baseline Characteristic and Skin Measurement Information of the Subjects of This Study

Characteristic				Average ± SD or *N* (%)
Population (*n*)				1,079
Gender (female, %)				100%
Age (years, mean ± SD)				40.81 ± 10.90
Phenotype (equipment)		Measure item	Code	Average ± SD or *N* (%)
Wrinkle (Primos CR)	Eye (right)	Average roughness (Ra)	W101	20.81 ± 5.24
		Maximum depth (Rmax)	W102	198.47 ± 71.97
	Glabella	Average roughness (Ra)	W103	25.18 ± 7.06
		Maximum depth (Rmax)	W104	193.75 ± 69.61
Moisture (Corneometer)	Glabella	Moisture content (A.U.)	A101	64.84 ± 9.97
	Cheek (right)	Moisture content (A.U.)	A102	70.13 ± 9.48
Melanin (Mexameter)	Pigmentation site	Melanin (M.I.)	M101	166.15 ± 35.83
	Nonpigmentation site	Melanin (M.I.)	M102	119.25 ± 28.35
Brightness (CM-700d)	Pigmentation site	Brightness (L*)	R201	59.95 ± 2.62
	Nonpigmentation site	Brightness (L*)	R202	63.22 ± 2.33
Oil (Sebumeter)	Glabella	Oil content ($\mu g/cm^2$)	L101	85.19 ± 61.23
	Cheek (right)	Oil content ($\mu g/cm^2$)	L102	48.57 ± 42.49
Sensitivity (10% lactic acid)		Sensitive	S101	534 (49.5%)
		Nonsensitive	S101	545 (50.5%)

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Table II
Summary of Significant Skin Phenotype Loci according to GWAS

SNP	Chr:BP	SNP cluster[a]	Minor allele	Annotation: Nearby gene (<500 kb)	Annotation: SNP Pos.	Annotation: eQTL gene	MAF: This study	MAF: KOR	MAF: ASN	β (SE)[b]	*P*
Wrinkle											
rs117381658	1:157,353,684	5	T	FCRL5	Downstream	—	0.07	0.04	0.01	0.952 (0.167)	1.52×10^{-8}
rs1961184	10:63,733,371	2	T	REEP3	Downstream	REEP3 (heart)	0.08	0.05	0.07	0.840 (0.164)	3.60×10^{-7}
rs1929013	1:244,230,708	15	G	ADSS	Downstream	—	0.34	0.35	0.33	−0.410 (0.092)	8.29×10^{-6}
rs7042102	9:92,001,508	4	T	SPTLC1	Downstream	SPTLC1 (skin)	0.41	0.44	0.45	0.392 (0.088)	9.30×10^{-6}
Moisture											
rs9873353	3:31,233,850	3	T	—	Intergenic	—	0.07	0.02	0.02	−0.567 (0.117)	1.47×10^{-6}
rs34567709	17:61,492,168	5	G	TBX4	Downstream	TBX4 (adrenal gland)	0.14	0.12	0.14	0.406 (0.086)	2.53×10^{-6}
rs1362404	16:51,973,264	3	G	—	Intergenic	—	0.24	0.28	0.29	−0.319 (0.069)	3.76×10^{-6}
rs7853290	9:71,638,804	1	A	TRPM3	Upstream	CEMIP2 (adipose)	0.08	0.07	0.09	0.533 (0.115)	3.96×10^{-6}
rs143938096	15:79,098,451	4	A	CTSH	Upstream	—	0.07	0.08	0.06	−0.534 (0.116)	4.40×10^{-6}
rs12955989	18:24,106,190	4	G	TTC39C	Intron 5	CABYR (artery)	0.20	0.21	0.15	0.338 (0.073)	4.57×10^{-6}
Pigmentation											
rs74653330	15:27,983,407	6	T	OCA2	Exon 15 (missense)	—	0.07	0.07	0.03	−1.092 (0.189)	1.04×10^{-8}
rs34466224	19:3,219,644	13	A	NCLN	Downstream	NCLN (skin)	0.22	0.22	0.22	0.602 (0.124)	1.33×10^{-6}
rs11685354	2:217,996,408	7	A	TNS1	Intron 1	RUFY4 (lung)	0.43	0.41	0.45	−0.477 (0.099)	1.82×10^{-6}
rs4653497	1:227,355,326	6	T	CDC42BPA	Upstream	CDC42BPA (skin)	0.49	0.49	0.44	0.446 (0.098)	6.51×10^{-6}
rs59784607	16:25,774,628	4	T	HS3ST4	Intron 1	—	0.21	0.19	0.13	−0.541 (0.121)	8.13×10^{-6}
rs76548385	7:1,291,682	10	T	UNCX	Downstream	—	0.09	0.09	0.07	0.768 (0.173)	9.92×10^{-6}
Oil											
rs308971	3:12,075,120	7	G	SYN2	Intron 1	SYN2 (skin)	0.24	0.26	0.19	−0.325 (0.071)	4.60×10^{-6}
rs151209785	18:74,549,791	8	C	CNDP1	Intron 1	—	0.07	0.03	0.02	−0.556 (0.121)	5.19×10^{-6}
rs9577919	13:113,861,036	1	T	GAS6	Intron 1	TMEM255B (skin)	0.06	0.05	0.07	0.598 (0.133)	7.92×10^{-6}
rs8107564	19:6,964,536	5	A	INSR	Downstream	—	0.11	0.11	0.09	0.430 (0.097)	9.67×10^{-6}
rs6490805	13:23,510,670	4	C	TNFRSF19	Upstream	—	0.18	0.16	0.15	0.352 (0.079)	9.84×10^{-6}

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Table II
Continued

SNP	Chr:BP	SNP cluster[a]	Minor allele	Annotation: Nearby gene (<500 kb)	Annotation: SNP Pos.	eQTL gene	MAF: This study	MAF: KOR	MAF: ASN	β (SE)[b]	*P*
SNP	Chr. pos	SNP cluster[a]	Minor allele	Annotation Nearby gene (<500 kb)	SNP Pos	eQTL gene	MAF This study	KOR	ASN	OR (95% CI)[c]	*P*
Sensitivity											
rs7334780	13:106,182,099	2	T		Intergenic	—	0.32	0.33	0.33	0.635 (0.525–0.768)	2.82×10^{-6}
rs41308	7:28,636,081	5	C	CREB5	Intron 4	—	0.36	0.36	0.35	1.564 (1.295–1.890)	3.57×10^{-6}

Chr: chromosome; BP: base pair; Pos: position; KOR: Korean; ASN: Asian; SE: standard error. The reference MAF was obtained from the KRGDB (http://coda.nih.go.kr/coda/KRGDB/index.jsp) and Ensembl DB (https://asia.ensembl.org).

[a]SNP cluster is the number of SNPs that satisfy $p < 0.05$ at ±100 kb around the top signal SNP indicated in GWAS.

[b]Calculated β and SE by linear regression.

[c]We analyzed OR using logistics regression. The SNPs with the top signals based on GWAS satisfying the cutoff value of $p < 5.0 \times 10^{-8}$ are underlined.

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IDENTIFICATION OF SNP GENOTYPE BASED ON SNP ARRAY

Oral swab samples were obtained, and DNA was extracted using the Exgene™ Tissue SV (GeneAll, Seoul, Korea). The Theragen Precision Medicine Research Array (PMRA) was customized and designed based on the Asian PMRA (Thermo Fisher Scientific, Waltham, MA) to obtain genetic variant information for 820,000 SNPs in the entire human genome. The Theragen PMRA array is an SNP array, which is the convergence of DNA hybridization, fluorescence microscopy, and solid-surface DNA capture. The SNP array has three major components: an allele-specific oligonucleotide probe, fragmented nucleic acid sequences labeled with fluorescent dyes, and a detection system that records and interprets the hybridization signal.

To identify 820,000 SNPs using the Theragen PMRAs, DNA was amplified, randomly broken into 25- to 125-bp fragments, purified, resuspended, and reacted with the Theragen PMRA to bind human genomic DNA to the fluorescently labeled allele-specific oligonucleotide probe. DNA that bound to the probe was hybridized to prepare for detection. Following hybridization, the bound targets were washed to remove unbound DNA fragments to minimize noise that results from nonspecific ligation events. We performed genotyping of 820,000 SNPs using the Theragen PMRA array according to the manufacturer's instructions. The array provides genome-wide coverage in five major populations (European, American, South Asian, East Asian, and African). In addition, the genotyping results, obtained with the array, confirmed that the accuracy of the 7.4 million markers in an Asian population, with minor allele frequencies (MAFs) >1% and >5% were 0.90 and 0.94, respectively.

To reduce potential concern about batch effects and the possibility of false associations, we applied highly stringent quality control measures, including assessment of DNA quality and probe binding, noise removal, confirmation of genotype accuracy, and correction of genotyping errors to select SNPs to use in the case and control datasets. The quality control procedures were performed for each of the 820,000 SNPs before the association tests were conducted. The SNP set was filtered based on the genotype call rates (≥0.95) and MAF (≥0.10). The Hardy–Weinberg equilibrium (HWE) was calculated for individual SNPs using an exact test. All the SNPs reported in this article exhibited HWE p-values > 0.01. After filtering, 560,795 polymorphic SNPs were analyzed on chromosomes 1 through 22.

ANNOTATION OF SNP-ASSOCIATED GENES

To identify and annotate genes that are functionally related to SNPs that show significance in GWASs, it was necessary to check the information of the SNP locus. Therefore, to identify the genes corresponding to the top p-value of the SNP associations in each analysis, SNP locus data were obtained from the UCSC Genome Browser (Genome Bioinformatics Group, University of Santa Cruz, Santa Cruz, CA). The gene annotations from the UCSC database and Genotype-Tissue Expression (GTEx) database (GTEx Analysis Release v. 8, http://www.gtexportal.org/, Cambridge, MA) were also used to predict the functional effects of the variants on transcripts. The GTEx database is provided by the GTEx project and provides the scientific community with resources to study the relationship between human gene expression and regulation and genetic variation. This project will collect and

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analyze multiple human tissues from donors who are densely genotyped to assess genetic variation within their genomes. By analyzing global RNA expression within individual tissues and treating the expression levels of genes as quantitative traits, variations in gene expression that are highly correlated with genetic variation can be identified as expression quantitative trait loci (eQTLs). Our gene expression data are based on this GTEx database.

STATISTICAL ANALYSIS

Three groups based on tertiles were delimited and scored based on the range of measurements and the number of samples for each skin phenotype. The total score was calculated by integrating the measured scores of the skin phenotypes (Table III). In addition, we performed association analysis through linear regression between the total score for the "target phenotype for GWAS" and the genetic variants, and the results of this analysis were adjusted by age. Most of the statistical analyses were performed using PLINK version 1.9 (https://www.cog-genomics.org/plink/) and SPSS (IBM SPSS Statistics Inc., New York, NY). *p*-values were not adjusted for multiple tests. $p < 1.0 \times 10^{-5}$ was considered statistically significant.

RESULTS

STUDY POPULATION AND THE RESULTS OF SKIN MEASUREMENTS

Table I summarizes the measurement information for the skin indicators wrinkles, moisture content, melanin/erythema, pigmentation, brightness, oil content, and sensitivity. In this study, we analyzed 1,079 subjects with a mean age of 40.81 years. The skin phenotypes were measured for each feature at two different sites, and the mean and standard deviation (SD) were recorded in units of the measuring devices. The average and SD values of the measurements for the five skin phenotypes of wrinkles, moisture content, pigmentation, oil content, and sensitivity are presented in Table I. In addition, we assigned codes to each measurement item to unify the different values for each phenotype and measurement instrument (Table I). In addition, we identified the distribution of the measurements for each code to identify trends in skin phenotypic changes with age (Supplementary Figure 1).

GWAS FOR EACH SKIN PHENOTYPE

We performed a GWAS of Korean women, in whom the skin traits wrinkles, moisture content, pigmentation, oil content, and sensitivity were measured (Table III). A total of 23 SNPs showed significant *p*-values ($p < 1.0 \times 10^{-5}$) according to the GWAS of the skin phenotype (Table II and Figure 1), which matched the MAFs in the reference database [Korean Reference Genome Database (KRGDB) http://coda.nih.go.kr/coda/KRGDB/index.jsp; Ensembl DB, https://asia.ensembl.org]. For the wrinkle phenotype, we found four SNPs, rs117381658 (*FCRL5*), rs1961184 (*REEP3*), rs1929013 (*ADSS*), and rs7042102 (*SPTLC1*), among which rs117381658 showed the most significant correlation ($\beta = 0.952$, $p = 1.52 \times 10^{-8}$) with skin phenotype changes for wrinkles (Figure 2A) and presented a

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Table III
Normalization and Classification of Skin Measurements

		Evaluation		Target phenotype for GWAS
Phenotype	Code	Tertile criteria	Total score formula	Total score range (min ~ max)
Wrinkle	W101	1: $0 \le x < 17.89$ 2: $17.89 \le x < 22.34$ 3: $22.34 \le x$	(W101 tertile) +	4–12
	W102	1: $0 \le x < 157.83$ 2: $157.83 \le x < 213.25$ 3: $213.25 \le x$	(W102 tertile) +	
	W103	1: $0 \le x < 21.19$ 2: $21.19 \le x < 27.75$ 3: $27.75 \le x$	(W103 tertile) +	
	W104	1: $0 \le x < 155.36$ 2: $155.36 \le x < 206.54$ 3: $206.54 \le x$	(W104 tertile)	
Moisture	A101	3: $0 \le x < 61.53$ 2: $61.53 \le x < 70.07$ 1: $70.07 \le x$	(A101 tertile) +	2–6
	A102	3: $0 \le x < 67.46$ 2: $67.46 \le x < 73.67$ 1: $73.67 \le x$	(A102 tertile)	
Pigmentation	M101	1: $0 \le x < 148.33$ 2: $148.33 \le x < 179.00$ 3: $179.00 \le x$	(M101 tertile) +	4–12
	M102	1: $0 \le x < 106.00$ 2: $106.00 \le x < 131.00$ 3: $131.00 \le x$	(M102 tertile) +	
	R201	3: $0 \le x < 58.81$ 2: $58.81 \le x < 60.93$ 1: $60.93 \le x$	(R201 tertile) +	
	R202	3: $0 \le x < 62.34$ 2: $62.34 \le x < 64.34$ 1: $64.34 \le x$	(R202 tertile)	
Oil	L101	1: $0 \le x < 52.00$ 2: $52.00 \le x < 92.33$ 3: $92.33 \le x$	(L101 tertile) +	2–6
	L102	1: $0 \le x < 27.00$ 2: $27.00 \le x < 51.00$ 3: $51.00 \le x$	(L102 tertile)	
Sensitivity	S101	1: nonsensitive, 2: sensitive		1 or 2

min: minimum; max: maximum.

cluster of five SNPs within ±100 kb that showed a significant correlation of $p < 0.05$ in the GWAS. The genes that showed an association were *FCRL3*, which mediates an inflammatory response; *REEP3*, which is involved in cell division and aging; *ADSS2*, which is involved in adenosine regulation; and *SPTLC1*, which is involved in maintenance of moisture and skin function. Moreover, in the case of rs117381658, a difference for the value was found as a result of measuring the wrinkle condition of the skin by genotype (Supplementary Figure 2). In particular, in the analysis of variance test of the grading scale value of wrinkle measurement, the wrinkle value increased according to the

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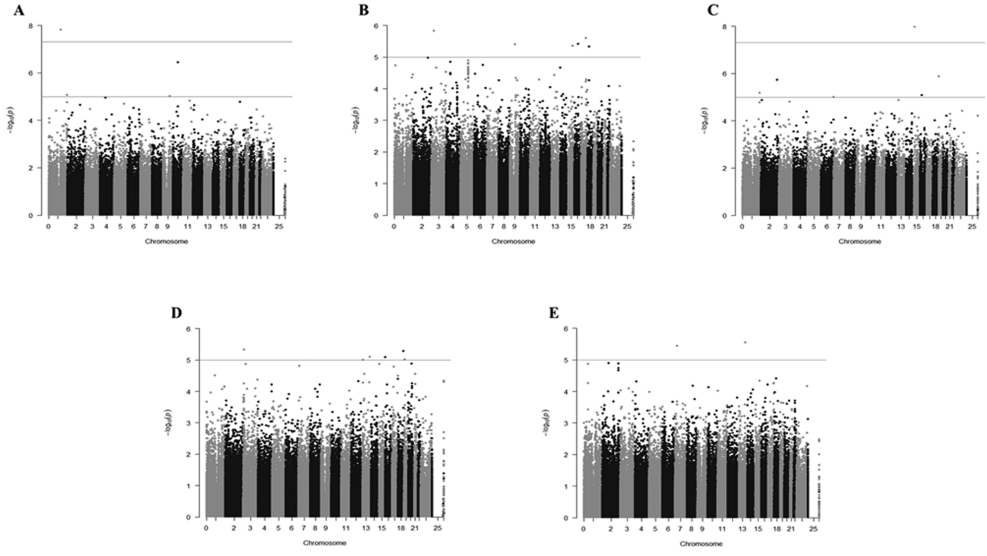

Figure 1. Manhattan plots of GWASs performed on skin phenotype. We performed genetic variation analysis of (A) wrinkles, (B) moisture content, (C) pigmentation, (D) oil content, and (E) sensitivity, and the results for each skin phenotype are represented in a Manhattan plot. In the plots, the *x*-axis represents the chromosome number; the *y*-axis represents the results of GWAS; the blue line indicates 1.0×10^{-5}, and the red line indicates 1.0×10^{-8} based on the *p*-value.

genotype ($p < 0.001$, Figure 2A). In addition, we found six SNPs that were associated with the pigmentation skin phenotype. In particular, rs74653330 showed correlations satisfying the strongest significance signal in the GWAS data ($\beta = -1.092$, $p = 1.04 \times 10^{-8}$), and six SNPs with $p < 0.05$ corresponding to the SNP cluster were also found. The rs74653330 SNP is located in the *OCA2* gene and is associated with diseases associated with skin pigmentation, such as albinism and melanoma. This gene is known to be involved in skin whitening and pigmentation. In the case of the strongest significance, the SNP rs74653330 was confirmed the change in the direct measurement value for pigmentation

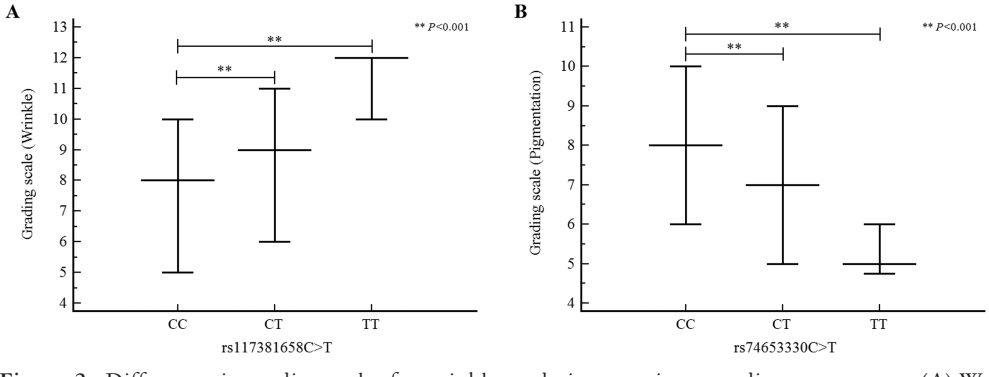

Figure 2. Differences in grading scales for wrinkles and pigmentation according to genotype. (A) We performed analysis of variance for the wrinkle grading scale with the *FCRL5* rs117381658 genotype and found significantly increasing values in CC versus CT or TT genotypes ($p < 0.001$). By contrast, (B) the pigmentation values were increasingly decreased with the *OCA2* rs74653330 genotype (CC vs. CT or TT genotypes; $p < 0.001$).

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levels (Supplementary Figure 3); moreover, the difference appeared as a result of confirming the difference by genotype in grading scale of the pigmentation measurement value ($p < 0.001$, Figure 2B). Although there were no strong signals for correlations with skin phenotypic changes, suggestive SNPs were identified for moisture content, oil content, and skin sensitivity. Regarding moisture content, correlations were identified for six SNPs, among which rs9873353 showed a strong correlation ($\beta = -0.567$, $p = 1.47 \times 10^{-6}$). However, there was no gene located near the SNP. We found five SNPs for oil content and two SNPs for skin sensitivity. The SNPs rs308971 ($\beta = -0.325$, $p = 4.60 \times 10^{-6}$) and rs7334780 (odds ratio = 0.635, $P = 2.82 \times 10^{-6}$) showed the highest association with the oil content and sensitivity of skin, respectively. When looking at the genes associated with these two SNPs, rs7334780 does not have a gene located nearby; however, rs308971 is located in the *SYN2 gene*, which is known to be involved in a neurotransmitter pathway. In particular, its function is associated with the lipid membrane in which neurotransmitters are embedded.

SKIN TISSUE EQTLS FOR SNPS IN THE GTEX PORTAL

When the GWAS-identified SNPs related to skin phenotypes were searched in the eQTL database (GTEx Portal, https://gtexportal.org/), differences in expression levels in skin tissues according to genotype were identified in five SNPs: rs7042102C > T, rs34466224 G > A, rs4653497T > C, rs308971 G > A, and rs9577919C > T (Supplementary Figure 4). In sun-exposed skin tissue, rs7042102C > T ($p = 5.2\times 0^{-12}$), rs34466224 G > A ($p = 1.1 \times 10^{-7}$), and rs308971 G > A ($p = 1.0 \times 10^{-13}$) showed significant differences in expression according to genotype, and rs4653497T > C ($p = 1.7 \times 10^{-4}$) and rs9577919C > T ($p = 4.9 \times 10^{-5}$) showed altered expression levels associated with genotype in non–sun-exposed skin tissue. The eQTL expression levels for each SNP change gradually with the minor allele homozygous genotype; rs7042102C > T, rs308971 G > A, and rs9577919C > T gradually increased in expression, and rs4653497T > C gradually decreased in expression. The difference in rs34466224 G > A expression level according to genotype was statistically significant, although it showed some deviation.

DISCUSSION

This study has numerous strengths compared with previous studies. First, most of the reported GWAS of skin phenotypes have been performed on pigmentation (25–27), sun sensitivity (28,29), and inflammation (30), whereas this study analyzed representative skin traits, such as wrinkles, moisture content, pigmentation, oil content, and sensitivity. Second, the analysis of the association between skin phenotypes and genes in Koreans is considered important because no such analysis has ever been performed in this population. Third, the sample size of the Korean participants was based on a large research group that included more than 1,000 people, and the statistical verification power was excellent. Because we generated an analytical dataset based on this group, our results are highly reliable. Finally, our results are meaningful in that the measured skin phenotype values were not analyzed based on individual measurements, but instead were averaged according to the measurement equipment and the measurement site, and scored by tertile. This approach applies universally to the data cleansing method generated through the analysis of the results

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because universality can be achieved by applying tertile-divided scoring to the measured values, even when using different equipment or measuring different areas.

There were also some limitations to our research. First, reproducibility was not verified in our study because no replication study exists. However, our findings provide scientific evidence, given the large sample size, that are considered to be reliable enough to include the *OCA2* SNP (rs74653330) (31,32), which is known to be associated with pigmentation.

As the results of these phenotypic studies show, there is a link between genetic variation and changes in skin characteristics which allows us to produce skin prediction models. If we can identify gene variants that affect skin phenotype changes, we can provide a personalized guide to apply to lifestyle, diet, and makeup and help prevent and resolve skin problems using customized cosmetics. By providing relief and therapeutic intervention in the early stages of symptoms, cosmetic problems and medical costs can be reduced. These results can help us understand the personality of each individual's skin and provide personalized cosmetics and skincare. In addition, we can also provide customized services based on genotype. We suggest that this study is a valuable foundation to produce customized cosmetics, wherein ingredients and materials may be added to the cosmetics for the prevention of skin damage or application of functional cosmetics, according to skin characteristic markers through genetic testing.

WRINKLES

Among the various skin traits examined, wrinkles were the primary skin phenotype showing the most obvious change. Generally, wrinkles appear naturally because of aging, which is an interesting field in cosmetic dermatology because it is a phenotype that does not recover completely, unlike scars or disease (33). However, wrinkles are not only caused by natural aging but can also be caused or accelerated by external factors and internal abnormalities (34). In particular, the internal immune response, triggered by environmental factors, can produce chronic inflammation and accelerate the occurrence of wrinkles (34). Aging results in the accumulation of the B-cell receptor, which increases the risk of chronic inflammation stimulated through this receptor and its induced transcript, *FCRL5* (35). FCRL3, a well-known member of the immunoglobulin receptor superfamily and one of several Fc receptor–like glycoproteins, has immunoreceptor tyrosine-based activation and inhibitory motifs in its cytoplasmic domain, which may play a role in the regulation of the immune system. According to our results, rs117381658 exists downstream of *FCRL5* and can affect *FCRL5* expression by forming a significant SNP cluster related to chronic inflammatory status (36). *FCRL5* expression levels can affect the inflammatory response and NF-κB, which can disrupt modulators of tissue homeostasis and affect skin aging (37). In addition, our study also identified SNPs associated with wrinkles, such as rs1961184 (*REEP3*), rs1929013 (*ADSS2*), and rs7042102 (*SPTLC1*). The SNP rs1961184 is located downstream of *REEP3* and has been reported to influence *REEP3* expression patterns in the heart tissue. *REEP3* is involved in GPCR signaling, affects the normal progression of mitosis, and is reported to be related to aging (38). Although there are no reports of *REEP3* directly impacting skin aging, it is a potential marker of skin aging. The SNP rs1929013 is located downstream of *ADSS2*, which encodes an enzyme that converts inosine monophosphate to adenosine monophosphate. Adenosine is a well-known factor involved in wrinkle improvement (39), and ADSS2 can regulate the function of adenosine (40). It is conceivable

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therefore that ADSS2 may have an effect on wrinkles. In addition, rs7042102, which is another SNP identified in this study, is a variant located downstream of the *SPTLC1*, for which its differences in expression, based on genotype in skin tissues, were confirmed in the eQTL database. Previous reports suggest that *SPTLC1* may be a causal gene that affects psoriatic lesions (41) and may also affect skin phenotype by functioning as a regulator of skin fibroblasts (42). *SPTLC1* is involved in the synthesis of sphingolipids, which are substances involved in maintaining the skin's barrier and moisture (43) and exerts skin improvement effects through skin moisturization and maintenance (44,45). From this perspective, *SPTLC1* may affect skin aging and the generation of wrinkles, if it causes abnormalities in skin maintenance.

MOISTURE CONTENT

The moisture content of the skin is closely related to the contraction of the stratum corneum. This outermost layer of the epidermis is important for the skin's barrier function because it has various proteolytic effects related to skin changes as well as to exfoliation, lipid synthesis, and inflammatory reactions (46). Dry skin and frequent inflammatory reactions in the stratum corneum may lead to diseases, such as atopic dermatitis and psoriasis. Internal factors involved in modulating the important characteristic of the skin moisture content include lipids of the stratum corneum, natural moisturizing factors, and external factors (47). However, there are still individual differences regarding changes in skin hydration, for which genetic variants have been highlighted as a likely explanatory factor and are currently being studied (15,48). Our results identified six SNPs, rs9873353, rs34567709, rs1362404, rs7853290, rs143938096, and rs12955989, which were associated with the skin moisture content. The SNP identified as the most significant marker of skin moisture in our results was rs9873353. This locus has several pseudogenes in the surrounding area, but no functional genes have been identified. However, there are SNPs that are considered to have other potential functions. Potential functional effects are suggested for rs7853290 and rs143938096, which are associated with the *CEMIP2* (*TMEM2*) and *CTSH* genes, respectively. Both of these genes can affect skin changes, especially *CEMIP2*, which regulates hyaluronan, a key component of physical functions of the skin, such as moisturization and lubrication (49). Regarding the differences in expression according to the SNPs in the eQTL database, although only adipose tissue eQTLs are included among the single-tissue eQTLs, the available multiple-tissue eQTLs confirmed that altered expression of *CEMIP2*, based on the rs7853290 genotype, also appears in the skin tissue. Therefore, it is possible that this SNP is capable of providing moisture and functionally controlling the barrier, which may explain our results.

PIGMENTATION

Changes in pigmentation are largely visible and can be found in the hair and eyes, in addition to local phenotypic variations in the skin. Pigmentation is the most studied topic among the various phenotypic variations of the skin and is caused by differences in melanin, where differences between individuals are caused by changes in the type, amount, or other parameters of melanin (13). These individual melanin differences are due to genetic

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characteristics, and many previous studies have reported melanin-related genes and gene mutations. In addition, differences and correlations between genes involved in pigmentation have been reported (17). In the present study, we identified new candidate loci, *TSN1*, *RUFY4*, *NCLN*, and *CDC42BPA*, as well as the previously reported *0CA2* gene, which is associated with various skin pigment-related diseases, such as albinism (13) and melanoma (50). In particular, an amino acid substitution (His615Arg) found in Asian populations has been reported to present at a high prevalence in cases of skin whitening and pigment changes (13). According to our results, rs74653330 is a missense (Ala481Thr) SNP that is re-associated with pigmentation in the East Asian population (31,32). Although the other genes are not functionally related to genes that directly affect melanin or skin tone, *TSN1* encodes collagen-associated intercellular adhesion proteins (51), and RUFY4 causes skin inflammatory responses (52). *NCLN* and *CDC42BPA* are not reported to be associated with pigmentation of the skin. However, our single-tissue eQTLs show differences in genotype expression in skin tissue. This suggests that our analysis and results are reproducible compared with previous studies, which indicates accuracy of our correlation model with skin measurements and suggests that we used effective analytical methods.

OIL CONTENT

Oil forms a protective film on the skin to prevent various problems, such as water evaporation and infection. However, when various factors result in abnormal control of oil content, problems such as inflammation, acne, and sebaceous gland hypersecretion may occur (53). In general, the amount of oil in the skin decreases with aging, which causes the skin to lose elasticity, moisture content, and dryness (54). In addition, recent studies have reported that skin diseases can be caused by excessive oil and that air pollutants may be absorbed by skin oil that can adversely affect skin health. As such, oil acts as a source of protection for the skin and prevents skin aging, in addition to preserving the moisture content (55). Therefore, rs308971, rs9577919, rs8107564, and rs6490805, which are associated with skin oil, may cause skin changes. The most significant SNP in our analysis was rs908971, which is located at intron 1 of the *SYN2* gene. Traditionally, SYN2 is associated with neurotransmitters (56). Recently, the association between the lipid metabolism and the *SYN2* gene has been reported (57). Based on this, *SYN2* is involved in the synthesis of synaptic vesicles and is associated with the formation of lipids and apolipoproteins (58), which may affect the formation of lipids in the body. There have also been reports of associations of SNPs with triglycerides (57), which may lead to associations in the lipid metabolism and further suggests that it is a process that can have a measurable effect on the skin. Furthermore, rs9577919 is located in intron 1 of *GAS6*, which mediates the inflammatory response and affects the development of psoriasis (59). In addition, rs8107564 is located downstream of the *INSR* gene. Insulin receptors encoded by *INSR* are involved in various mechanisms, including the regulation of inflammation (60), cancer development (61), and keratinocyte proliferation (62). Although rs6490805 shows a correlation with skin oil content, there are no relevant research reports or reported functional correlations. However, because single-tissue eQTLs show differences in expression levels in skin tissue according to genotype, potential oil abnormalities can be inferred from observed skin changes. rs7334780, the SNP with the strongest significance in sensitivity of the skin, is located in the intergenic region, and there is no gene present nearby; therefore, further studies are needed to determine its function.

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