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Original Article

Genome-wide scan identified genetic variants associated with skin aging in a Chinese female population

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ABSTRACT

Background: The progression of human skin aging has a strong genetic basis. However, recent studies have mainly focused on Caucasian populations and we have thus performed a genetic association study on skin aging signs in Han Chinese population.
Objective: To investigate genetic risk factors in skin aging in Han Chinese female, we performed a genome-wide association study.
Methods: We collected genotype data from 1534 Han Chinese female from Taizhou cohort and evaluated 15 skin aging phenotypes by using the validated skin aging SCINEXA™ score. Genetic associations were tested by linear and logistic regression analyses and adjusted for potential confounders.
Results: Six genomic regions significantly associated with a risk for skin aging were revealed : 6q24.2 (rs3804540, $P = 4.6 \times 10^{-9}$, additive model) with size of pigmented spots on forehead, 10q26.13 (rs4962295, $P = 1.9 \times 10^{-8}$, additive model) with wrinkles under eyes, 15q21.1 (rs28392847, $P = 1.6 \times 10^{-8}$, additive model) with crow's feet, 2p25.1 (rs191497052, $P = 5.5 \times 10^{-9}$, dominant model) with telangiectasia, 13q34 (rs3825460, $P = 3.7 \times 10^{-8}$, dominant model) with size of pigmented spots on cheeks and 16p13.11(rs76053540, $P = 5.0 \times 10^{-9}$, dominant model) with nasolabialfold. The signal on 15q21.1 was replicated in the meta-analysis with two independent Caucasian cohorts ($P = 8.6 \times 10^{-10}$). We have also successfully replicated in our cohort an association between SNP rs1048943 of gene *CYP1A1* ($P = 7.1 \times 10^{-4}$) and pigmented spots on cheeks previously described in Caucasian cohort.
Conclusions: Our study has identified new genetic risk factors for signs of skin aging in the Han Chinese. This study suggests there are differences in genetic susceptibility to skin aging between Caucasians and the Han Chinese.

1. Introduction

During the recent years, skin aging has attracted more and more attention as a part of proper aging, which includes pigmented spots, wrinkling, sagging and telangiectasia [1]. Previous research has pointed out that East Asian skin was less wrinkled and showed less but larger pigmented spots than Caucasians [2–4]. Besides, it was also reported that the epidermal gene expression is different according to the ethnical background [3]. Genome-wide association studies have found a series of skin aging signs associated with

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intrinsic genetic factors, including *SLC45A2*, *IRF4*, *MC1R*, *ASIP*, *BNC2*, *VASH2*, *GNG2*, *STXBP5L*, and *HLA-C* [5–9]. However, these studies have mainly focused on the Caucasian population, and no genetic study on skin aging in the Han Chinese has previously been performed.

To the best of our knowledge, we present here the first genome-wide association study on skin aging phenotypes in the Han Chinese females. To validate our findings, we then performed a replication analysis in two independent Caucasian cohorts.

2. Materials and methods

2.1. Study population

Our discovery set included 1534 the Han Chinese females from Taizhou, Jiangsu Province in China. These participants were sampled as part of the Taizhou Longitudinal Study, and detailed characteristics have been described before [10]. Questionnaire and blood samples were collected from these healthy Chinese women ranging from 31 to 86 years, and all participants provided written informed consent. This research program was conducted with the approval of the Ethics Committee of Fudan University (Ethics Research Approval No.85), Shanghai, China and followed the principles of the Declaration of Helsinki.

As previously reported, the fragmentation of collagen type I and skin damage from lifetime sun exposure have a significant difference between women younger and older than 50 years [11,12]. We thus selected females older than 50 as a second discovery dataset comprising 1084 females.

Our replication sets consist of two Caucasian cohorts. The first one includes 502 females from the SU.VI.MAX cohort, a longitudinal cohort study, conducted in middle-aged French adults [13]. The second one includes 462 German females from the SALIA cohort. A detailed description of the SALIA study can be found in Schikowski et al. [14].

2.2. Assessment of skin aging phenotypes

Both assessments of skin aging phenotypes in the Taizhou and in the SALIA cohorts were performed according to a validated skin aging score system, the SCINEXA™ (Score of intrinsic and extrinsic skin aging [1], Supplementary Figs. 1–12). The laxity of cheeks and eyelids are identified as intrinsic skin aging items while pigmented spots, coarse wrinkles, telangiectasis, solar elastosis and cutis rhomboidalis nuchae are known as extrinsic skin aging signs. The double-blind evaluation was carried out on each individual, and the average scores of two assessors were used in the following analysis. The skin aging phenotypes used in SU.VI.MAX cohort including photoaging, lentigine, sagging and wrinkling were determined independently by three dermatologists from reference photographs [15].

2.3. Genotype quality control and imputation

All SNPs were genotyped using the microarray Illumina Human OmniZhonghua8 V1.1 895k. A total of 894,517 SNPs were included. In the GWAS stage, case samples with a call rate lower than 98% were excluded. We excluded samples with ambiguous sex by the check-sex function of PLINK [16]. We determined identity-by-descent (IBD) similarity using PLINK, estimated the cryptic relatedness for each pair of samples, and excluded individuals with excess autosomal heterozygosity (PI_HAT>0.33), as were duplicates or 1st degree relatives identified using IBS probabilities. Furthermore, we also excluded individuals with an excess of heterozygosity indicated by an inbreeding coefficient below -0.2 or above 0.2. After removing unqualified individuals, 1534 samples were kept. Then for SNPs, we excluded SNPs with a call rate lower than 98%, a minor allele frequency smaller than 1% in all samples, or a p value of deviation from Hardy-Weinberg equilibrium (PHWE) less than 0.001. We also removed SNPs with no chromosome information and SNPs that each pair has one identical rs number of the same physical position in the same chromosome. After application of quality-control criteria, there were 802,328 remaining SNPs and 1534 females to be analyzed.

The imputation was done by IMPUTE2 [17] using 1000 genome phase 3 as a reference [18], and was pre-phased using SHAPEIT2 [19–21]. The imputed data here is analyzed as the best guess. The thresholds were 0.8 and SNPs with imputation quality less than 0.8 were considered missing. Imputed variants with a call rate lower than 98%, a minor allele frequency smaller than 1% in all samples, or a p value of deviation from Hardy-Weinberg equilibrium (PHWE) less than 0.001 were excluded in the further analyses. 6,328,611 SNPs were kept after imputation.

2.4. Population stratification analysis

The effects of possible population stratification were corrected using the EIGENSTRAT [22] tool from the EIGENSOFT package. To this end, Taizhou Longitudinal Study (TZL) data was combined with our data for Uygur (UYG) and 1000 Genomes Phase 3 data for YRI, CHB and CEU populations. 102,284 SNPs in low linkage equilibrium (r^2<0.2) were selected for analysis. Principal component (PC) analysis did not find any outliers in TZL (Supplementary Fig. 13).

2.5. HLA imputation

As reported by Jia [22], classic HLA alleles were imputed at HLA classI (*HLA-A*, *HLA-B*, *HLA-C*) and classII (*HLA-DPB1*, *HLA-DQB1*, *HLA-DRB1*) using SNP2HLA and a reference panel from the Han-MHC panel [23] that included 10,689 of individuals of Chinese descent. We used software "fcgene" to perform the quality control and alleles with r^2 higher than 0.9, SNP-HWE lower than 1×10^{-6}, MAF bigger than 0.05, missing rate less than 0.1 were kept for association analysis. A comparison of imputed HLA alleles to 4-digit HLA sequencing data available for the Han Chinese dataset [23] showed high concordance: the correlation of HLA alleles was 0.983 (P value $< 2.2 \times 10^{-16}$). In all, 97 classic HLA alleles and 1143 SNPs in HLA region were successfully imputed and available for analysis.

2.6. Statistical analysis and functional annotation

The genome-wide association analysis was performed by multivariate linear regression (PLINK software) in additive, dominant, and recessive models across genotyped and imputed SNPs. Correction of the quantitative trait comparisons for multiple SNPs was performed in PLINK by permutation testing ($n = 1.0 \times 10^9$). A meta-analysis of discovery dataset and two replication datasets was performed on the results of linear association analysis using the meta-analysis function in Metal [19]. For all analyses, age, BMI, sun exposure, smoking, the degree of education, type of fossil fuel used for cooking, hormonal status and top 10 PCs were taken as covariables. The P-values were adjusted by the Bonferroni correction (statistical threshold = 5×10^{-8}) and false discovery rate (FDR).

The functional annotation for signal SNP was visualized on the UCSC Genome Browser. DHS, TFs, Histone mark annotation (H3K27ac, H3K4me1, H3K4me3) peaks for the normal human lung fibroblasts were obtained from the ENCODE database [20]. The expression quantitative trait loci (eQTL) information of non-coding SNPs was taken from the GTEx database [24] (www.gtexportal.org/home/).

3. Results

3.1. Characteristics of study populations and their clinical skin aging classification

This genome-wide association scan was performed in two datasets: 1534 the Han Chinese females and a subset of 1084 females older than 50. These datasets were sampled from the Taizhou cohort, China in 2014. For the whole female dataset, the average age and BMI was 55.9 and 21.7. 96.9% of these people have never smoked, and the majority of them are post-menopausal (67.1%). They spent an average of 2.1 h under sun exposure per day, and few people have a junior college or higher education (6.6%). Compared with the whole female dataset, females older than 50 years have achieved a lower education and they are otherwise comparable with regards to sun exposure time, smoking and type of fossil fuel used for cooking. The detailed description is given in Table 1.

We have collected skin aging phenotypes by applying SCINEXA. The wrinkle, pigmented spots, laxity, solar elastosis, telangiectasis and cutis rhomboidalis nuchae were assessed for all of 1534 females and the elderly female. There was a significant difference between two groups in all 15 skin aging phenotypes. Telangiectasis and cutis rhomboidalis nuchae are more severe in group of all female, while other 13 skin aging phenotypes are more severe in group of female older than 50. Table 2 shows the mean score and p value of each trait.

3.2. Genome wide associations with skin aging phenotypes

The association analysis between 15 skin aging phenotypes and genotyped and imputed SNPs was performed by using linear regressions and factors described in Table 1 were used as covariates. Genome-wide significance was obtained for three genomic regions for the additive model, located at 6q24.2 (size of pigmented spots on the forehead, females older than 50, rs3804540: $P = 4.6 \times 10^{-9}$, genotyped; Fig. 1), 10q26.13 (wrinkles under eyes, all females, rs4962295: $P = 1.9 \times 10^{-8}$, genotyped; Fig. 1) and 15q21.1 (crow's feet, all femalse, rs28392847: $P = 1.6 \times 10^{-8}$, genotyped; Fig. 1). QQ plots showed that no inflation was found in GWAS analysis (Supplementary Figs. 14, 15). For the dominant model, three other genomic regions besides 10q26.13 were found, which located at 2p25.1 (telangiectasia, females older than 50, rs191497052: $P = 5.5 \times 10^{-9}$, imputed; Fig. 2), 13q34 (size of pigmented spots on cheek, all females, rs3825460: $P = 3.7 \times 10^{-8}$, imputed; Fig. 2) and 16p13.11 (nasolabial fold, females older than 50, rs76053540: $P = 5.0 \times 10^{-9}$, imputed; Fig. 2). No significant result with other skin aging phenotypes or with the recessive model was found. Detailed information on the significant signals is presented in Table 3 To further confirm the reliability of skin aging associated variants, we performed the replication analysis in a sample set of middle-aged French women from the SU.VI.MAX cohort and another independent German set from SALIA, including 502 and 462 women, respectively. SNP rs28392847, which is associated with crow's feet, was successfully replicated in the French population ($P = 1.7 \times 10^{-3}$, Table 3). By performing the meta-analysis of three datasets, we found SNP rs28392847 on 15q21.1 have a more significant p value ($p = 8.6 \times 10^{-10}$, Table 3). This result indicates the solid association between SNP rs28392847 with crow's feet. The p value of other two SNPs, rs4962295 and rs76053540, did not reach the significant threshold but also have a p value of 10^{-6} (Table 3). This may due to the difference between skin aging associated genetic factors in East Asian and Caucasian. The three other SNPs were not available in the two Caucasian replication cohorts for they are specific for the Asian population in 1000 Genome, and we did not get meta-analysis result for them.

3.3. Linkage disequilibrium and functional annotations of SNP rs28392847

In order to check the functionality of SNP rs28392847, we analyzed the rs28392847 genotypes in function of crow's feet, regional association plot and functional annotations. People with G

Table 1
Description of the study population.

Sample size		All female (n = 1534)	Female older than 50 (n = 1084)
Age[years]	Mean(SD)	55.9(9.3)	60.0(5.5)
	Min-Max	31-86	50-86
Body mass index (BMI) [kg/m2]	Mean(SD)	21.7(9.4)	24.5(5.5)
	Min-Max	15.6-50.2	16.4-42.1
Sun exposure (Average hours outside per day under sun exposure after age of sixteen)	Mean(SD)	2.1(1.4)	2.2(1.4)
	Min-Max	0.5-10	0.5-10
Smoking			
Never smoked	%Yes(n)	96.9(1589)	96.3(1044)
Ex-smokers	%Yes(n)	1.5(25)	1.6(17)
Current smokers	%Yes(n)	1.6(26)	2.1(23)
Passive smoking	%Yes(n)	62.7(1104)	62.0(672)
Education			
Primary school or lower education	%Yes(n)	37.9(666)	48.0(306)
Junior high school	%Yes(n)	17.4(305)	28.6(310)
Senior high school	%Yes(n)	34.9(613)	20.0(216)
Junior college or higher education	%Yes(n)	6.6(116)	3.5(38)
Type of fossil fuel used for cooking			
Electricity/gas only	%Yes(n)	85.6(1473)	83.2(902)
Both electricity/gas and coal/biomass	%Yes(n)	12.3(212)	14.9(161)
Coal/biomass only	%Yes(n)	2.1(35)	1.9(21)
Hormonal status			
Menopause	%Yes(n)	67.1(1174)	91.5(992)

The description of the two datasets is shown above, and the information of the whole female dataset is recorded on the left while the elderly female is on the right.

Table 2
Description of skin aging phenotypes.

Skin aging phenotypes	All female, AM(sd) (n = 1534)	Female older than 50, AM(sd) (n = 1084)	Statistical difference between two groups in skin aging phenotypes
Size of pigmented spots on forehead	0.78(0.82)	0.97(0.92)	$1.7\times10^{-9***}$
Number of pigmented spots on forehead	0.49(0.42)	0.59(0.44)	$3.5\times10^{-11***}$
Size of pigmented spots on cheeks	1.39(1.05)	1.71(1.13)	$2.2\times10^{-16***}$
Number of pigmented spots on cheeks	0.84(0.49)	1.02(0.48)	$2.2\times10^{-16***}$
Wrinkles on forehead	2.54(1.09)	2.70(1.12)	$4.2\times10^{-7***}$
Frown lines	2.54(1.14)	2.96(1.07)	$2.2\times10^{-16***}$
Crow's feet	2.30(0.85)	2.53(0.74)	$2.2\times10^{-16***}$
Wrinkles under eyes	2.48(0.79)	2.61(0.71)	$1.4\times10^{-9***}$
Wrinkles on upper lip	1.76(0.83)	2.10(0.82)	$2.2\times10^{-16***}$
Nasolabial folds	2.69(0.83)	2.96(0.73)	$2.2\times10^{-16***}$
Teleangiectasis	0.94(0.83)	0.88(0.79)	0.01^{**}
Laxity of eyelids	2.77(1.25)	3.18(1.08)	$2.2\times10^{-16***}$
Laxity of cheeks	2.60(1.09)	2.91(0.98)	$2.2\times10^{-16***}$
Solar elastosis	0.34(0.55)	0.43(0.61)	$4.4\times10^{-9***}$
Cutis rhomboidalis nuchae	0.74(0.54)	0.66(0.47)	$3.2\times10^{-6***}$

Abbreviations: AM(sd), arithmetic mean (standard deviation).
The description of the two datasets is shown aboveand the information of the whole female dataset is recorded on the left while the elderly female is in the middle. The statistical difference between two groups in skin aging phenotypes was calculated by using *t*-test and the p value is listed in the right side. * $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

allele of SNP rs28392847 have a more severe crow's feet sign compared with the A allele (Fig. 3A). For a lower frequency of G allele in the Han Chinese than Caucasian (68% in the Han Chinese and 90% in Caucasian), our result is consistent with a report of a reduced crow's feet in the Han Chinese compared with Caucasian [4]. The regional association plot for the rs28392847 is shown in

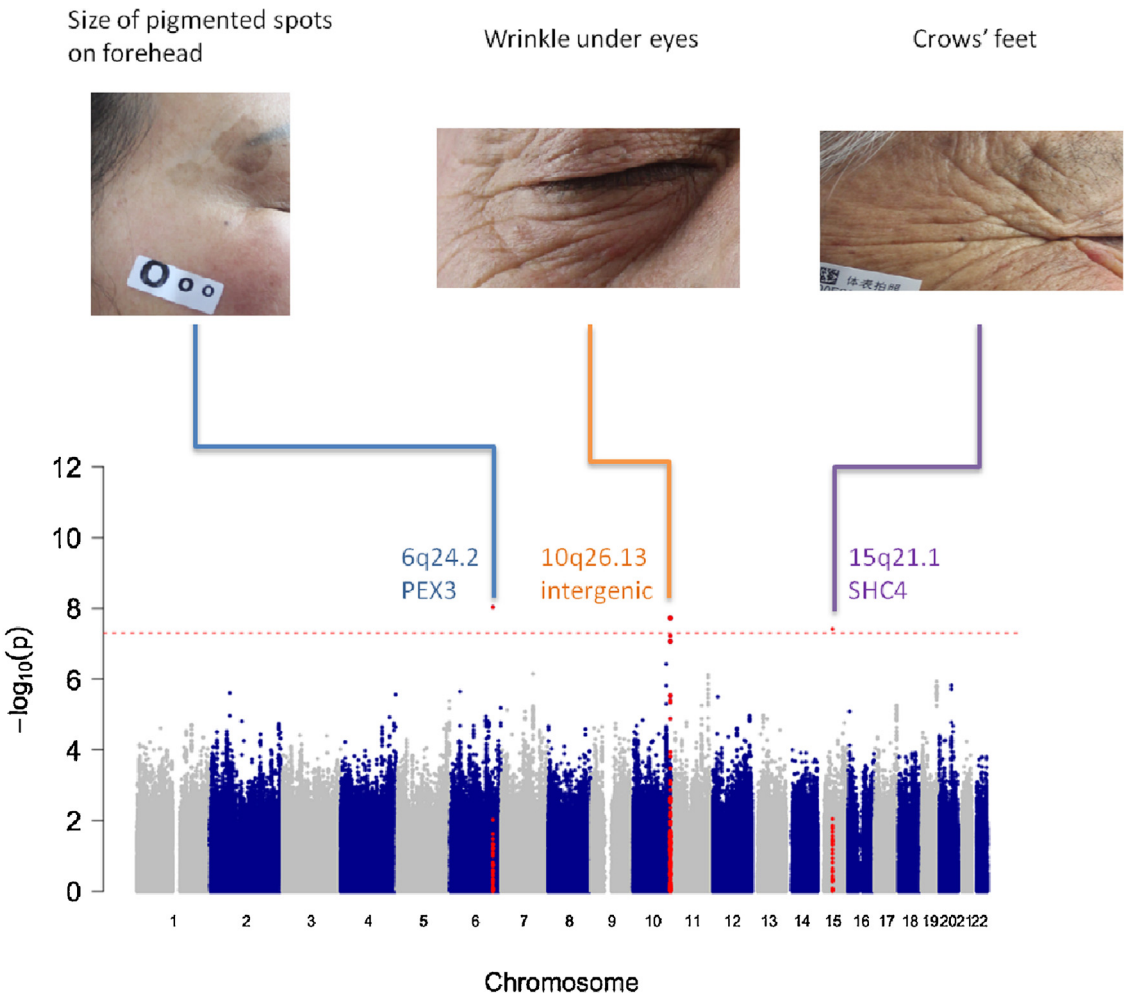

Fig. 1. Manhattan plot of the association study with skin aging phenotypes from additive model. Distribution of –log10 P obtained for the associations from additive model tested between the genotypes and skin aging phenotypes. Three phenotypes listed above were the size of pigmented spots on the forehead, wrinkle under eyes and crow's feet from left to right. The red line represents P = 5.0 × 10-8. Age, BMI, sun exposure, smoking, the degree of education, type of fossil fuel used for cooking.

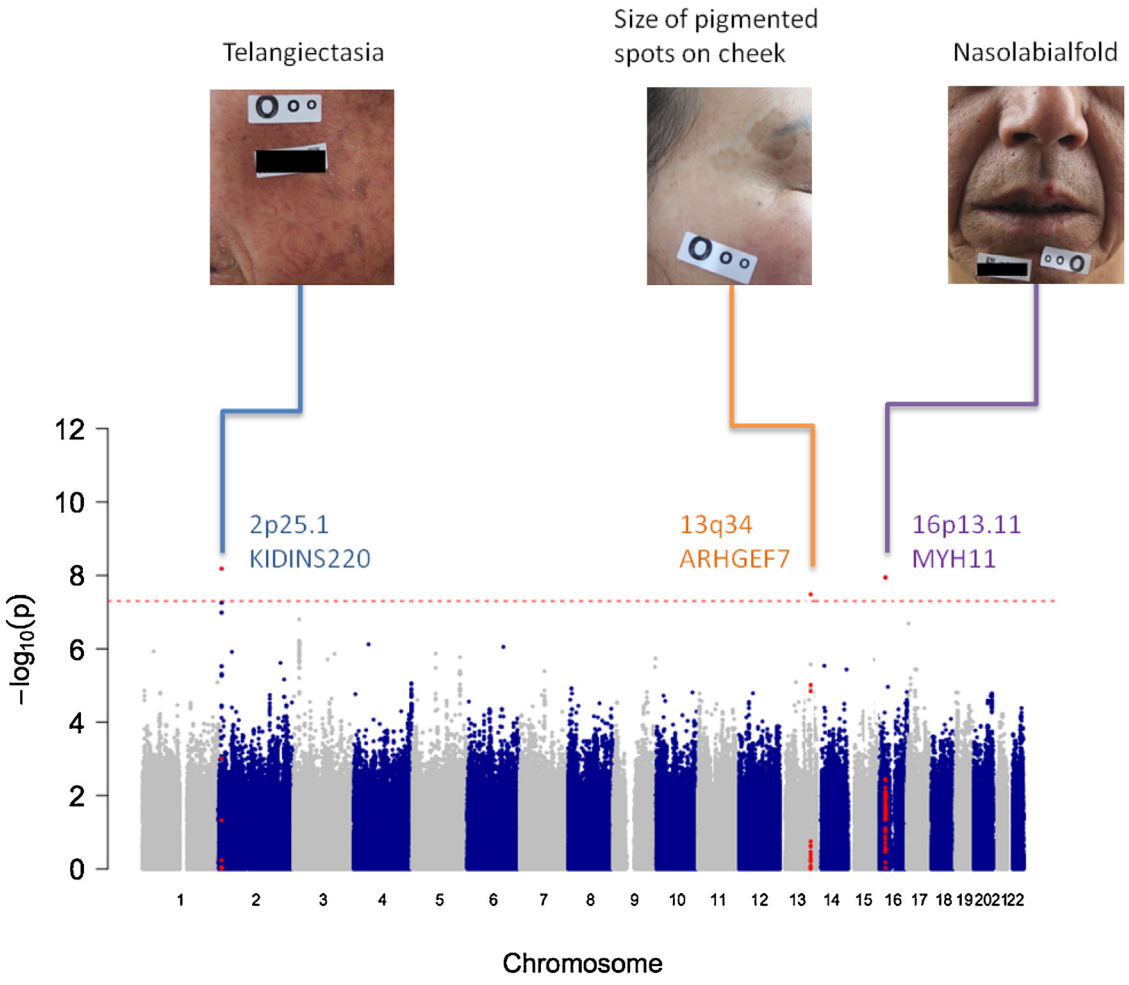

Fig. 2. Manhattan plot of the association study with skin aging phenotypes from dominant model. Distribution of –log10 P obtained for the associations from dominant model tested between the genotypes and skin aging phenotypes. Three phenotypes listed above were telangiectasia, size of pigmented spots on cheek and nasolabialfold from left to right. The red line represents P = 5.0 × 10-8. Age, BMI, sun exposure, smoking, the degree of education, type of fossil fuel used for cooking.

Fig. 3B, and there is only little linkage disequilibrium between marker SNP and others.

Given that SNP rs28392847 is highly associated with crow's feet in both the Han Chinese and French women, we performed the functional annotation of SNP rs28392847, including Histone modification, DNaseI hypersensitivity sites and transcription factors (TF) binding sites. The result is shown in Fig. 3C. Few functional annotations were found for SNP rs28392847. We also checked the expression quantitative trait loci (eQTL) information for rs28392847 in the GTEx database and found no result. This finding indicated that rs28392847 might not act as a causal variant but rather a SNP in LD with the causal variant in the formation of crow's feet.

3.4. Association of HLA and other candidate SNPs with skin aging phenotypes

We checked whether there was any classical HLA gene associated with skin aging phenotypes in the Han Chinese females and found no significant variant passing the Bonferroni threshold (the same as GWAS analysis, $p = 5\times 10^{-8}$). Thus, there may be no strong association between HLA SNPs and skin phenotypes in the Han Chinese females.

We also selected genes for xenobiotic metabolism and antioxidative system due to their association to skin aging and environmental exposures as well as genes associated with any of the 15 skin aging phenotypes by previous GWAS performed in Caucasians: there were 21 previously reported candidate SNPs covering 10 of the 15 phenotypes (Supplementary Table 1). We looked for the potential associations in the two discovery datasets (all females and females older than 50). We only found the SNP rs1048943 in the *CYP1A1* gene (cytochrome P450 family one subfamily A member 1) significantly associated with pigmented spots on cheeks ($P = 7.1 \times 10^{-4}$), but no other SNP passed Bonferroni corrected threshold (Supplementary Table 2). The minor allele, C, of SNP rs1048943 showed a positive effect on the score of pigmented spots on cheeks (effect = 0.07) and the grade of this skin aging phenotype increased by 0.07 for each copy of this allele. For females older than 50, there were no association (Supplementary Table 3).

4. Discussion

This is the first genome-wide study on 15 skin aging phenotypes in the Han Chinese females and we identified six signals passing genome-wide significance. Among them, a signal on the *SHC4* gene was also replicated in an independent French sample set. *SHC4* gene was previously found to be able to subvert the *EGFR* trafficking [25] and is linked for the first time to crow's feet. *EGFR* plays a vital role in the formation of the epidermis, and this may explain the association found between *SHC4* gene and crow's feet. The top SNP of the pigmented spots associated region at 6q24.2,

Table 3
Association results for significant SNPs in the discovery dataset with skin aging phenotypes.

SNP	Locus	Position	Gene	Location	Fitting model	Phenotype	Study	MAF	P value	Effect
rs3804540	6q24.2	143779148	*PEX3*	Intron	Additive	Size of pigmented spots on forehead	Female older than 50	0.04	4.6×10^{-9}	0.60
							1st Replication	Not Available		
							2nd Replication	Not Available		
							Meta analysis	Not Available		
rs4962295	10q26.13	125392657		Intergenic	Additive	Wrinkle under eyes	Whole female	0.06	1.9×10^{-8}	−0.26
							1st Replication	0.22	0.17	−0.06
							2nd Replication	0.2	0.25	0.09
							Meta analysis		2.8×10^{-6}	−0.22
rs28392847	15q21.1	49248001	*SHC4*	Intron	Additive	Crow's feet	Whole female	0.32	1.6×10^{-8}	−0.16
							1st Replication	0.1	1.7×10^{-3}	−0.31
							2nd Replication	0.1	0.45	0.07
							Meta analysis		8.6×10^{-10}	−0.21
rs191497052	2p25.1	8977814	KIDINS220	Promoter	Dominant	Telangiectasia	Female older than 50	0.03	5.5×10^{-9}	0.60
							1st Replication	Not Available		
							2nd Replication	Not Available		
							Meta analysis	Not Available		
rs3825460	13q34	111955942	ARHGEF7	Exon	Dominant	Size of pigmented spots on cheek	Whole female	0.07	3.7×10^{-8}	0.53
							1st Replication	Not Available		
							2nd Replication	Not Available		
							Meta analysis	Not Available		
rs76053540	16p13.11	15927276	MYH11	Intron	Dominant	Nasolabialfold	Female older than 50	0.22	5.0×10^{-9}	−0.27
							1st Replication	Not Available		
							2nd Replication	0.25	0.41	−0.13
							Meta analysis		3.4×10^{-6}	−0.20

Whole female: Han Chinese sample of 1534 females collected in Taizhou.
Female older than 50: Han Chinese sample of 1084 females older than 50 collected in Taizhou.
1st Replication: French sample of 502 females from SU.VI.MAX cohort.
2nd Replication: German sample of 462 females from SALIA cohort.
Meta analysis: Meta analysis of Taizhou cohort, SU.VI.MAX cohort and SALIA cohort.

rs3804540, is located in the intron of *PEX3*. The product of this gene is involved in peroxisome biosynthesis and integrity. Mutation of the *PEX3* gene leads to numerous peroxisomal membrane structures in fibroblasts [26] and this may explain the relationship between *PEX3* gene and pigmented spots. Finally, SNP rs4962295 (10q26.13) located between *RPS27P18* and *MRPS21P6*, rs76053540 located in the intron of *MYH11*, rs191497052 located in the promoter of *KIDINS220*, and rs3825460 located in the exon of *ARHGEF7* were found to be associated respectively with wrinkles under eyes, nasolabial fold, telangiectasia, and size of pigmented spots on cheeks. No biological interpretation can be provided for these associations at present. To validate the reliability of our significant signals, we have also calculated FDR adjusted p value and permutation test of all six significant associations. We found that all six SNPs have a p value smaller than 0.05 after FDR adjustment or $5\times\ 10^{-8}$ for permutation test (Supplementary Table 4), suggesting they are less likely to be false positives due to multiple testing.

By performing meta-analysis with two Caucasian datasets, SNP rs28392847 had a more significant p value while the other two, rs4962295 and rs76053540, only had a p value of 10^{-6}. This result indicates that these two signals may be false positives, or this may due to the different impact of genetic factors in different populations. Also, our signals detected from dominant model included two SNPs with MAF less than 0.1 (rs191497052, MAF = 0.03; rs3825460, MAF = 0.07). Neither of the two SNPs were replicated. So there is indeed a possibility that these two signals could be false positive due to the relatively lower MAF and limited sample size. These results need caution to interpret, and should be tested in larger sample size in the future.

Skin aging phenotypes of the SU.VI.MAX cohort, based on evaluations of the full face, included number of pigmented spots, wrinkles, and laxity. In the Han Chinese and SALIA cohorts, SCINEXA was used to evaluate pigmented spots, wrinkles, and laxity of specific facial areas. Here we used the full face evaluation of pigmented spots, wrinkles, and laxity in SU.VI.MAX to replicate the significant results of corresponding phenotypes of specific facial areas measured by SCINEXA. It is not uncommon to have slightly different phenotypes between discovery and replication studies. Even if the wrinkling measuring methods were slightly different, we still successfully replicated the signal found in crow's feet. It's true that this may lead to the false negative, but we believe that a signal replication will be more solid.

While we have identified six new genomic regions associated with skin aging phenotypes in the Han Chinese females, we have also investigated previously reported SNP associations with skin ageing in other cohorts. We could confirm in our study an association between rs1048943 in exon of the *CYP1A1* gene and pigmented spots on cheeks. As previously reported, different populations may have different associations between skin aging phenotypes and genetic variants. While genes *IRF4*, *MC1R*, *ASIP* and *BNC2* influence pigmented spots in Caucasians from Holland [5], the genome-wide association of pigmented spots in Japanese identified the genes *PPARGC1B* and *RAB11FIP2* [27]. This underlines the importance of performing genome-wide association study on the same skin aging phenotype in different populations.

Among the six significant signals associated with skin aging phenotypes, only one of them was replicated in Caucasian samples. Previous studies have shown that the parameters in Caucasian skin types are different from the one in the Han Chinese and the epidermal gene expression is also different [2,3]. The non-replication of our other findings in Caucasian samples may be caused by a false positive or more likely by the difference in ethnical background. Our sample size included about 1534 individuals and, as for any study, it may be insufficient to eliminate the risk of false positive signals. In order to solve this problem, we

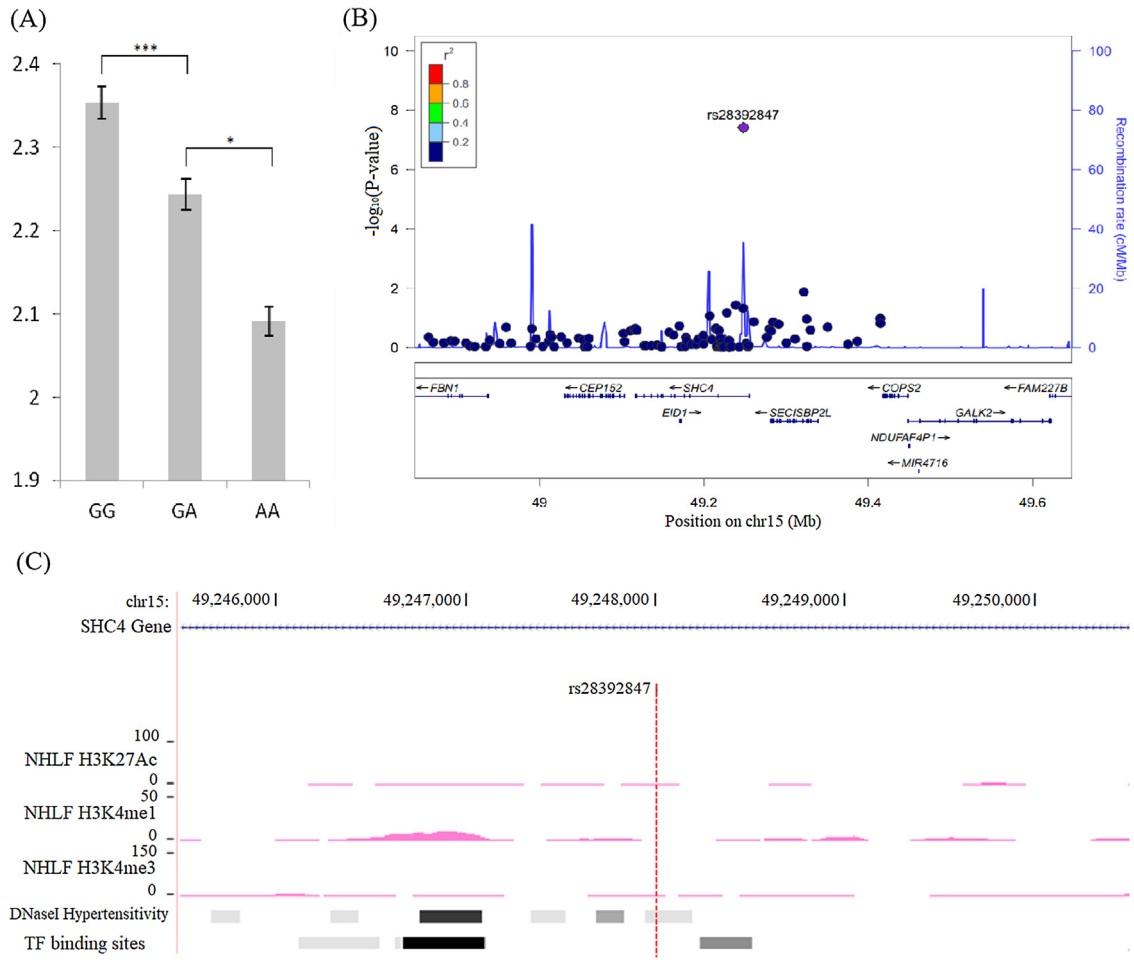

Fig. 3. The phenotypic correspondence histograms and functional annotations for SNP rs28392847. (A) The phenotypic correspondence histograms between different genotypes of SNP rs28392847. *t*-test was performed (* $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$). (B) Regional association plot for rs28392847. Results (-log10 P) are shown in the region flanking 400 kb on either side of the marker SNP. The marker SNP was shown in purple, and the different colors indicate r2 values of the rest of SNPs. (C) Functional annotation of SNP rs28392847. Histone modifications (H3K4me1, H3K4me3, and H3K27Ac) in the normal human lung fibroblasts (NHLF) are shown. DNaseI Hypersensitivity in 125 cell types from ENCODE and transcription factor (TF) binding sites are from UCSC Genome Browser track. The red dashed vertical line indicates the position of the SNP rs28392847.

need to collect more samples to increase the statistical power of the genome-wide association study in our future work and validate our result in larger sample sets.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.08.010.

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