Genetic ancestry, skin pigmentation, and the risk of cutaneous squamous cell carcinoma in Hispanic/Latino and non-Hispanic white populations

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Although cutaneous squamous cell carcinoma (cSCC) is one of the most common malignancies in individuals of European ancestry, the incidence of cSCC in Hispanic/Latinos is also increasing. cSCC has both a genetic and environmental etiology. Here, we examine the role of genetic ancestry, skin pigmentation, and sun exposure in Hispanic/Latinos and non-Hispanic whites on cSCC risk. We observe an increased cSCC risk with greater European ancestry \((P = 1.27 \times 10^{-42})\) within Hispanic/Latinos and with greater northern \((P = 2.38 \times 10^{-65})\) and western \((P = 2.28 \times 10^{-49})\) European ancestry within non-Hispanic whites. These associations are significantly, but not completely, attenuated after considering skin pigmentation-associated loci, history of actinic keratosis, and sun-protected versus sun-exposed anatomical sites. We also report an association of the well-known pigment variant \(Ala111Thr\) \((rs1426654)\) at \(SLC24A5\) with cSCC in Hispanic/Latinos. These findings demonstrate a strong correlation of northwestern European genetic ancestry with cSCC risk in both Hispanic/Latinos and non-Hispanic whites, largely but not entirely mediated through its impact on skin pigmentation.

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Cutaneous squamous cell carcinoma (cSCC) is one of the most common malignancies among individuals of European ancestry, and, in recent years, its incidence has been increasing not only in non-Hispanic whites but also in Hispanics and Asians\(^{1-2}\). However, a thorough analysis of cSCC susceptibility across different ethnic groups is lacking, and data on clinical characteristics of cSCC in patients of Hispanic, Asian, or African ancestry are limited\(^{6,7}\).

Lighter skin pigmentation has been shown to be a strong determinant of cSCC risk because it provides less protection against damage from ultraviolet radiation (UVR) exposure compared to darker skin pigmentation\(^{8,9}\). For example, individuals from northern Europe, who are more likely to manifest light skin color, have higher incidence rates of cSCC as compared to their southern European counterparts, who have a higher prevalence of darker skin color\(^{10}\). Genetic variation is an important contributor to variation in skin pigmentation and is also a risk factor for cSCC\(^{11,12}\). Variants in a number of pigmentation-related genes, including SLC45A2, IRF4, TYR, ASIP, OCA2, HERC2, and MC1R, have been associated with the risk of cSCC in populations of European ancestry\(^{13-19}\), supporting the presumed biological importance of lighter pigmentation in susceptibility to cSCC. To our knowledge, the role of skin pigmentation and the effect of skin pigmentation loci on cSCC susceptibility has not been investigated in other populations.

Genetic ancestry studies have shown that the correlation between allele frequency variation and geographic distances enables the identification of an individual’s geographic/ethnic ancestry\(^{20-22}\). By estimating the genetic ancestry of individuals, it is possible to determine whether geographic variation in disease prevalence, such as that observed for cSCC in European and Hispanic/Latino populations, is correlated with genetic ancestry. Variation in skin pigmentation has also been associated with genetic ancestry\(^{23}\), which could explain variation in the risk of cSCC both within and between European descent populations and other populations. To date, no study has examined the role of genetic ancestry and the risk of cSCC.

To address these gaps, we conduct genetic ancestry analyses of cSCC susceptibility in Hispanic/Latino and non-Hispanic white ethnic groups from the large multiethnic Genetic Epidemiology Research in Adult Health and Aging (GERA) cohort. Next, we determine whether genetic ancestry associations are entirely due to genetically predicted skin pigmentation using skin pigmentation probabilities generated by the HIrisPlex-S program\(^{24-26}\) based on 36 known skin pigment genetic variants. We then determine whether genetic ancestry associations are modified by sun exposure by considering actinic keratosis (AK) diagnosed by a physician as a clinical marker for chronic UVR exposure and assessing whether observed genetic ancestry associations are comparable at sun-protected anatomical sites and with non-cutaneous SCC (ncSCC).

## Results

**GERA cohort and cSCC risk.** The study sample consisted of 11,396 cSCC cases and 86,186 controls from four ethnic groups (non-Hispanic whites, Hispanic/Latinos, East Asians, and African-Americans) (Table 1) in the GERA cohort. Among GERA participants, cSCC cases were more likely to be male (54.8%) and non-Hispanic white (96.8%). The prevalence of cSCC was much higher among non-Hispanic whites (14.0%) compared to Hispanic/Latinos (3.5%), East Asians (0.8%), and African-Americans (0.4%). Because of the limited number of cases among East Asians and African-Americans, we focused on the non-Hispanic white and the Hispanic/Latino GERA subjects for the subsequent study analyses.

**Genetic ancestry and cSCC risk.** We examined genome-wide genetic ancestry using principal components (PCs) that were calculated within each race/ethnicity group separately. Within Hispanic/Latinos, the first two PCs were geographically interpretable, with PC1 representing greater European versus Native American ancestry and PC2 representing greater African versus European ancestry\(^{21}\). Both PC1 and PC2 were associated with cSCC risk within Hispanic/Latino GERA participants, as illustrated in Fig. 1a. We identified significant associations between cSCC risk and PC1 (Model 1: \(\beta = 122.48, P = 1.27 \times 10^{-42}\)), as well as PC2 (\(\beta = 43.52, P = 6.44 \times 10^{-33}\)) (Table 2 and Supplementary Table 1), indicating that individuals with greater European ancestry had a higher risk of cSCC compared to those with greater Native American and/or African ancestry.

Within non-Hispanic whites, the first two PCs also represented geographically interpretable genetic ancestry, with PC1 characterizing a northwestern versus southeastern Europeancline and PC2 a northeastern versus southwestern Europeancline\(^{21}\). Both PC1 and PC2 were strongly associated with cSCC risk within non-Hispanic white GERA participants, as illustrated in Fig. 1b. We identified significant associations between cSCC risk and PC1 (Model 1: \(\beta = 30.12, P = 2.38 \times 10^{-42}\)), as well as PC2 (\(\beta = -25.23, P = 2.28 \times 10^{-49}\)) (Table 2 and Supplementary Table 2), indicating that individuals of northwestern European ancestry had the highest cSCC risk.

**Genetic ancestry and skin pigmentation.** To determine the degree to which genetic ancestry associations with cSCC were due to genetically determined skin pigmentation, we predicted skin color for each study participant using HIrisPlex-S, a program that utilizes 36 known skin pigmentation genetic variants (Supplementary Table 3) to generate probabilities of different pigment traits\(^{24-26}\). Out of the 36 single-nucleotide polymorphisms (SNPs) that have been reported to be associated with skin pigmentation, we excluded from further analysis four SNPs (rs3212355, rs1805006, rs11547464, and rs1110400) that were not polymorphic (minor allele frequency (MAF) < 1.0%) in either GERA non-Hispanic whites or Hispanic/Latinos.

The genetic ancestry associations with cSCC were strongly but incompletely attenuated after including HIrisPlex-S skin pigmentation predictions (Model 2: \(\beta = 67.31, P = 1.77 \times 10^{-9}\) for PC1 and \(\beta = 28.43, P = 0.072\) for PC2 in Hispanic/Latinos; \(\beta = 16.53\), ...

### Table 1 Characteristics of the cSCC cases and controls from GERA cohort.

| Characteristic                | cSCC cases | cSCC controls |
|------------------------------|------------|---------------|
| Sex                          |            |               |
| Female                       | 5149 (8.9%)| 52,488        |
| Male                         | 6247 (15.6%)| 33,698      |
| Ethnicity                    |            |               |
| Non-Hispanic White           | 11,028 (14.0%)| 67,555     |
| Hispanic/Latino              | 294 (3.5%)| 8196         |
| East Asian                   | 61 (0.8%)| 7292         |
| African American             | 13 (0.4%)| 3143         |
| Prior AK                     |            |               |
| Yes                          | 7461 (32.3%)| 15,624      |
| No                           | 3935 (5.3%)| 70,562       |
| Tumor location (N patients)  |            |               |
| Sun-exposed only             | 9996       |               |
| Sun-protected only            | 420        |               |
| Both                         | 465        |               |
$P = 2.42 \times 10^{-19}$ for PC1 and $\beta = -19.36, P = 5.33 \times 10^{-29}$ for PC2 in non-Hispanic whites (Table 2 and Supplementary Tables 1 and 2). Within Hispanic/Latino, dark/dark to black skin color showed strong variations in prevalence by geographic ancestry. Within non-Hispanic whites, we also observed variation in skin pigment by geographic ancestry, with lighter skin more prevalent in the northwest of Europe and dark skin more prevalent in the southeast of Europe (Supplementary Figs. 1 and 2).

Many pigmentation SNPs (17/30 = 56.7%) were associated with cSCC risk in the GERA meta-analysis (combining non-Hispanic whites and Hispanic/Latinos) at Bonferroni significance ($P < 0.05 / 30 = 0.00167$), including 1 SNP at BNC2 and 5 at OCA2/HERC2, and among those, 11 reached genome-wide level of significance: 2 at SLC45A2, 1 at IRF4, 2 at TYR, 4 at 16q24.3 (DEF8-MC1R), and 2 at RALY (Supplementary Data 1), consistent with previous GWAS of cSCC\cite{18,19}. Three additional SNPs (i.e., 1 at TYRP1 and 2 at 16q24.3) reached nominal significance ($P < 0.05$).

**Table 2** Associations between genetic ancestry and cSCC risk in GERA non-Hispanic whites and Hispanic/Latinos.

|                      | Non-Hispanic whites | Hispanic/Latinos |
|----------------------|---------------------|------------------|
| **Model 1: Ancestry** (age, sex, and PCs as covariates) | | |
| PC1 30.12 (1.76) | $2.38 \times 10^{-65}$ | 122.48 (8.95) | $1.27 \times 10^{-42}$ |
| PC2 $-25.23$ (1.71) | $2.28 \times 10^{-49}$ | 43.52 (15.97) | $6.44 \times 10^{-3}$ |
| **Model 2: Model 1 and HIrisPlex-S skin pigmentation predictions** | | |
| PC1 16.53 (1.84) | $2.42 \times 10^{-19}$ | 67.31 (11.19) | $1.77 \times 10^{-9}$ |
| PC2 $-19.36$ (1.73) | $5.33 \times 10^{-29}$ | 28.43 (15.80) | 0.072 |
| Intermediate skin $-1.32$ (0.05) | $6.97 \times 10^{-150}$ | $-2.23$ (0.31) | $1.54 \times 10^{-12}$ |
| Dark skin $-2.24$ (0.017) | $3.47 \times 10^{-41}$ | $-3.22$ (0.39) | $1.09 \times 10^{-16}$ |
| **Model 3: Model 2 and genetic risk score** | | |
| PC1 17.20 (1.84) | $1.07 \times 10^{-20}$ | 56.54 (11.49) | $8.54 \times 10^{-7}$ |
| PC2 $-18.33$ (1.75) | $4.41 \times 10^{-26}$ | 28.44 (15.63) | 0.069 |
| Intermediate skin $-0.68$ (0.06) | $5.94 \times 10^{-26}$ | $-1.31$ (0.37) | $4.54 \times 10^{-4}$ |
| Dark skin $-1.46$ (0.17) | $4.44 \times 10^{-17}$ | $-2.14$ (0.46) | $2.93 \times 10^{-6}$ |
| GRS 0.09 (0.01) | $8.50 \times 10^{-54}$ | $0.13$ (0.03) | $1.26 \times 10^{-5}$ |
| **Model 4: Model 3 and sun exposure** | | |
| PC1 11.49 (1.90) | $1.52 \times 10^{-9}$ | 41.30 (11.98) | $5.67 \times 10^{-4}$ |
| PC2 $-12.99$ (1.80) | $5.37 \times 10^{-13}$ | 18.67 (15.17) | 0.22 |
| Intermediate skin $-0.45$ (0.07) | $1.26 \times 10^{-11}$ | $-0.94$ (0.39) | 0.016 |
| Dark skin $-0.96$ (0.18) | $4.84 \times 10^{-8}$ | $-1.62$ (0.47) | $5.54 \times 10^{-4}$ |
| GRS 0.07 (0.01) | $1.14 \times 10^{-29}$ | $0.12$ (0.03) | $6.84 \times 10^{-5}$ |
| Prior AK 1.39 (0.02) | $<2.23 \times 10^{-308}$ | $1.64$ (0.14) | $5.12 \times 10^{-30}$ |

Note: In Models 2, 3, and 4, the HIrisPlex-S prediction for the combination of very pale skin with pale skin served as the reference group. Each model was adjusted for age, sex, and additional PCs. We also included the percentage of Ashkenazi (ASHK) ancestry as a covariate for the non-Hispanic white analyses. PC principal component, $\beta$ beta, SE standard error, GRS genetic risk score (based on 14 SNPs previously reported to be associated with cSCC risk), AK actinic keratosis.
Genetic ancestry and known cSCC-associated loci. To determine whether known cSCC-associated loci explain the remaining genetic ancestry associations (after considering genetically determined skin pigmentation), we repeated the ancestry analysis, including a genetic risk score (GRS) based on 14 reported cSCC-SNPs (as two other reported SNPs, rs192481803 and rs74899442, were not polymorphic (MAF < 1.0%) in the GERA Hispanic/Latino and non-Hispanic white subjects, and so were excluded from the GRS and subsequent analyses) (Supplementary Table 4 and Methods online). The 14 SNPs were confirmed to be associated with cSCC risk in GERA at Bonferroni significance (P < 0.05/14 = 0.0036) (Supplementary Data 2). When including the GRS in addition to the HIrisPlex predicted skin pigments, the ancestry associations were slightly attenuated in Hispanic/Latinos (Model 3: $\beta = 56.54$, $P = 8.54 \times 10^{-4}$ for PC1 and $\beta = 28.44$, $P = 0.069$ for PC2) but were not attenuated further in non-Hispanic whites (Model 3: $\beta = 17.20$, $P = 1.07 \times 10^{-20}$ for PC1 and $\beta = −18.33$, $P = 4.41 \times 10^{-26}$ for PC2) (Table 2 and Supplementary Tables 1 and 2). Thus, the genetic ancestry effects in Hispanic/Latinos and non-Hispanic whites cannot be explained completely by current cSCC-SNPs.

Shared genetic risk loci for cSCC and skin pigmentation traits. Of the 14 reported cSCC-SNPs and the 56 known skin pigmentation genetic variants, 5 SNPs were overlapping: IRF4 rs12203592, T YR rs1126809, OCA2 rs1800407, MC1R rs1805007, and R ALY rs6059655. Further, several known SNPs to influence skin pigmentation or cSCC risk were located within the same locus and were in strong linkage disequilibrium (LD), including rs28777 and rs35407 at SLC45A2 (R2 = 0.98, $D’ = 1$), rs12913832 and rs12916300 at HERC2 (R2 = 0.84, $D’ = 0.96$), and rs8051733 and rs4268748 at DEF8 (R2 = 0.73, $D’ = 0.97$) (Supplementary Table 5). This suggests that those SNPs represent the same signal at those loci and that those SNPs are most likely proxies of causal variants influencing both skin pigmentation and cSCC risk. It is noteworthy that rs683 at T YRP1, known to influence skin color pigment but not previously associated with cSCC risk, showed suggestive association with cSCC risk in our GERA non-Hispanic white sample (P = 0.0064) (Supplementary Data 1). T YRP1 encodes the melanosomal enzyme tyrosinase-related protein 1, and mutations in this gene can cause albinism and confer increased risk for familial cutaneous melanoma.27-29

Of the 14 cSCC-SNPs, five (35.7%) were not associated with HIrisPlex-S skin pigment probabilities in GERA (non-Hispanic whites or Hispanic/Latinos) (P ≥ 0.05), including SNPs at FOXP1, T PRG1/TP63, H LA-DQA1, A HR, and SEC16A loci (Supplementary Data 3). Of the five cSCC-SNPs that were not associated with HIrisPlex-S skin pigment probabilities, four (other than SEC16A rs57994353) were strongly associated with genetic ancestry PC1 or PC2 (Supplementary Table 6). That is, genetic risk factors other than those that affect pigment are also responsible for the association of ancestry and cSCC risk.

Independent signals for cSCC at skin pigmentation loci. To determine whether there were additional independent signals at the skin pigmentation loci for cSCC risk, we conducted conditional analyses of the skin pigmentation loci for cSCC association by including all the 14 known cSCC-SNPs in the regression model. We found independent signals at four known skin pigment loci among non-Hispanic whites (i.e., SLC45A2, HERC2, MC1R, and DEF8) (Supplementary Data 4). Most of these SNPs have been previously reported to be associated with pigmentation traits, including rs16891982 at SLC45A2, rs1667394 at OCA2/HERC2, and rs1805008 at MC1R.

Local ancestry. To determine whether each of the 16 known cSCC- and/or skin pigmentation-associated loci show evidence of increased European ancestry in cSCC cases compared to controls, we conducted local ancestry analyses in GERA Hispanic/Latinos using a method for local ancestry inference, which leverages the structure of LD in the ancestral population (LAMP-LD).33 After correction for testing multiple loci, we detected a significant association between cSCC risk and local European genetic ancestry at SLC24A5, where on average cases were of 91.8% European descent compared with controls at 3.8% (P = 1.71 × 10^{-4}).

SNP prioritization and annotations. To identify which genetic variants within the 18 cSCC and/or skin pigmentation loci (14 cSCC + 4 conditional hits at known skin pigmentation loci) are likely to be causal, we computed each variant’s ability to explain the observed signal and derived the smallest set of variants that included the causal variant with 95% probability.34 In each of the 18 loci, the corresponding 18 credible sets contained from 1 to 3868 variants in Hispanic/Latinos (7391 total variants, Supplementary Data 5) and from 1 to 1104 variants in non-Hispanic whites (1373 total variants, Supplementary Data 6). Interestingly, two variants (i.e., IRF4 rs12203592 and SLC45A2 rs16891982) had >50% probability of being causal in both Hispanic/Latinos and non-Hispanic whites. Additional five variants (i.e., TYR rs1126809, R ALY rs6059655, B N2C-CTN LN rs74664507, DEF8 rs4268748, and HERC2 rs12916300) had >50% probability of being causal in non-Hispanic whites.

Genetic ancestry associations and sun exposure. To determine whether chronic UVR/sun exposure mediates the observed genetic ancestry associations, we repeated the ancestry analysis, including AK as a covariate. In GERA, among cSCC cases, 65.5% had AK (Table 1). When including AK in addition to the HIrisPlex predicted skin pigments and the GRS, the ancestry associations were attenuated further but not eliminated (Model 4: $\beta = 41.30$, $P = 5.67 \times 10^{-4}$ for PC1 and $\beta = 18.67$, $P = 0.22$ for PC2 in Hispanic/Latinos; $\beta = 11.49$, $P = 1.52 \times 10^{-9}$ for PC1 and
As a secondary analysis, we assessed genomic ancestry associations with cSCC arising in anatomical sites exposed to the sun compared to sun-protected anatomical sites. Among cSCC cases, 87.7% had tumors at sun-exposed sites only compared to 3.7% for sun-protected only, and 4.1% for both (Table 1). While we found significant associations between genomic ancestry and cSCC risk at anatomical sites exposed to the sun ($\beta = 127.68, P = 2.11 \times 10^{-39}$ for PC1 and $\beta = 72.29, P = 8.69 \times 10^{-4}$ for PC2 in Hispanic/Latinos; $\beta = 30.62, P = 1.16 \times 10^{-40}$ for PC1 and $\beta = -25.82, P = 4.83 \times 10^{-46}$ for PC2 in non-Hispanic whites), the effects of these associations were reduced but not eliminated at anatomical sites unexposed to the sun (Model 1; $\beta = 47.93, P = 0.094$ for PC1 and $\beta = -5.27, P = 0.78$ for PC2 in Hispanic/Latinos; $\beta = 20.37, P = 0.0094$ for PC1 and $\beta = -17.07, P = 0.024$ for PC2 in non-Hispanic whites) (Supplementary Fig. 3 and Supplementary Table 8). After adjusting for skin pigment traits, the genomic ancestry associations with cSCC at anatomical sites unexposed to the sun were attenuated further and no longer significant (Model 2; $\beta = 37.84, P = 0.32$ for PC1 and $\beta = -7.16, P = 0.71$ for PC2 in Hispanic/Latinos; $\beta = 9.83, P = 0.23$ for PC1 and $\beta = -11.85, P = 0.12$ for PC2 in non-Hispanic whites).

### Discussion

In this large multiethnic study, we found that the risk of cSCC was associated with genomic ancestry within Hispanic/Latinos and non-Hispanic whites. Specifically, a greater risk of cSCC was associated with higher European (compared to Native American or African) ancestry among Hispanic/Latinos and northern and western European ancestry among non-Hispanic whites. After considering genomic variants known to influence skin pigmentation or cSCC risk and considering AK, which is a marker of chronic sun exposure, these genomic ancestry associations were significantly attenuated but not eliminated. Moreover, the associations between genomic ancestry and cSCC risk were attenuated but not eliminated in cases where the cSCC arose in sun-protected anatomical sites compared to the sun-exposed anatomical sites. Finally, we did not observe significant associations between genomic ancestry and ncSCC risk.

These findings suggest that genetically determined skin pigmentation can explain much of the ancestry effects in both Hispanic/Latinos and non-Hispanic whites and that additional loci associated with genomic ancestry remain to be discovered. However, we found evidence for an ancestry effect on the non-pigment genomic loci associated with cSCC. This suggests that these additional cSCC loci that remain to be discovered may or may not be associated with skin pigmentation and the genomic ancestry effect may extend beyond just skin pigmentation, per se.

This is, to our knowledge, the first study to examine genomic risk factors for cSCC in Hispanic/Latino populations, and we identified significant associations between the risk of cSCC and genomic ancestry, genetic predictors of skin pigmentation, sun exposure, and cSCC risk loci previously identified in populations of European descent. We also found evidence that the percentage of European ancestry at SLC24A5 was strongly correlated with cSCC case status ($P = 1.71 \times 10^{-4}$) in Hispanic/Latinos. cSCC cases contained on average 91.8% European ancestral alleles at the SLC24A5 locus compared to 3.8% in controls, suggesting that European-specific variation may contribute to cSCC risk at this locus. SLC24A5 is a well-known target of positive selection, associated with lighter skin color in admixed populations. Indeed, we showed that the well-known amino acid substitution at this locus, Ala111Thr, previously shown to be a
dichotomous between Europeans and the rest of the world, and strongly associated with pigment in populations in which it is polymorphic (e.g., Hispanics and South Asians)\textsuperscript{37–39}, is also a risk factor for cSCC in our Hispanic/Latino subjects. This is an important novel cSCC association uniquely found in this population, to date. Future studies can investigate whether this variant may also be an important risk factor in African-American populations.

Among non-Hispanic whites, we observed that a higher cSCC risk is associated with northern European genetic ancestry as compared to southern European ancestry; however, we also found a greater risk of cSCC in subjects with northwestern European ancestry compared to northeastern European ancestry. Observational studies have reported a north–south gradient of cSCC susceptibility in European countries with cSCC incidence rate rising with increasing latitude\textsuperscript{10}. This latitudinal gradient, which is paradoxically inversely correlated with ultraviolet light indices, a known risk factor for cSCC, is thought to be a result of the gradient of skin color observed in Europe, as the proportion of individuals with darker skin types increase with decreasing latitude\textsuperscript{10}. After considering the effect of skin pigmentation, the ancestry associations were significantly attenuated but not eliminated. This suggests that skin pigmentation is an important contributor to cSCC risk and the European latitude gradient, but does not fully explain it.

We recognize several potential limitations of our study. First, any association with genetic ancestry may represent not only variation in genetic risk factors but also other factors that correlate with genetic ancestry, such as environmental or socio-cultural factors\textsuperscript{40,41}, and so it is possible that the residual ancestry associations that we observed may reflect non-genetic risk factors for cSCC. Second, the skin pigmentation traits that we used in the current study were based on HHisPlex-S probabilities estimated using genetic information, as direct pigment measures are not available in the GERA cohort. This may result in underestimates of the effects of pigment variation due to phenotype misclassification. However, HHisPlex-S system has been shown to predict pigment traits accurately when using all the required SNPs for skin color\textsuperscript{24}, with average prediction accuracy of 91% for skin color. Third, we used a clinical surrogate for cumulative lifetime exposure to UV, namely clinician-diagnosed AKs, which could be a possible intermediate step in the development of cSCC. We also recognize that specifying tumor locations according to sun exposure may depend on individual behavior, which could affect the generalizability of our sun-exposed versus sun-protected findings. However, our findings from ncSCC, which are consistent with these results, are unlikely to be affected by either of these limitations. Finally, certain analytical strata of our study have relatively few cases (e.g., low number of Hispanic/Latino cases compared to non-Hispanic whites, and low number of sun-protected cases) that may limit our power to detect significant associations in these cases.

Our study also has important strengths. It is based on a unique and very large multiethnic cohort of individuals, who were all members of a single integrated health delivery system. Further, this is the first study that enabled cross populations comparison (i.e., between Hispanic/Latino and non-Hispanic whites) by taking into account genetic ancestry and known risk factors for cSCC.

In conclusion, we reported variation in cSCC risk not only across populations, but also within Hispanic/Latino and non-Hispanic white populations. This variation in cSCC risk is due in part to genetic variation in skin pigmentation genes and its interaction with sun exposure leading to higher risk. These results, based on additive models, underscore the importance of incorporating genetic ancestry, skin pigmentation traits, and sun exposure into genetic analyses of skin cancer. However, additional cSCC genetic risk loci and other cSCC risk factors that correlate with genetic ancestry remain to be discovered. Thus, our study highlights the importance of considering genetic structure when mapping the genetic basis of a disease phenotype showing correlation between prevalence and geographic origin. Our findings identifying genetic ancestry as an important risk factor for cSCC enable identification of the most vulnerable members of a population at high risk for cSCC and can lead to prevention strategies for this highly prevalent cancer\textsuperscript{42–44}.

### Methods

#### Study population

Cancer of skin squamous cell carcinoma. Potential eligible study subjects were adult GERA cohort participants who had no diagnostic codes for rare genetic disorders associated with increased cSCC risk\textsuperscript{45}. cSCC cases were defined as subjects whose pathology records were consistent with incident cSCC (invasive or in situ, excluding angenital and mucosal SCCs). Controls were subjects with no pathology records consistent with cSCC. After quality control (QC) was applied to all potential eligible subjects, a total of 97,582 subjects were eligible for analysis, consisting of 11,396 cSCC cases and 86,186 controls from four ethnic groups (80.6% non-Hispanic white, 8.7% Hispanic/Latino, 7.5% East Asian, and 3.2% African American).

#### Non-cutaneous squamous cell carcinoma.

The KPNC (Kaiser Permanente Northern California) Cancer Registry captured all SCC cases in the GERA cohort. We identified individuals as nSCC cases by excluding individuals with cSCC from the overall SCC case group. Controls were GERA participants who were neither cSCC nor nSCC cases.

#### History of AK.

AK is a clinical surrogate of cumulative lifetime exposure to UV. In a large, population-based study of skin cancer, the presence of AKs correlated most strongly with the variables estimating the cumulative number of hours spent outdoors in a self-administered questionnaire\textsuperscript{46}.

All study procedures were approved by the Institutional Review Board of the KPNC Institutional Review Board. Written informed consent was obtained from all participants.

#### Anatomic site of tumor.

Anatomic locations of the cSCC were classified according to the potential of the anatomic area to be chronically exposed to the sun, rather than more likely to be covered by clothing, as follows: sun-exposed (head, neck, hands, feet, and legs) and sun-protected (abdomen, buttocks, and hips).

#### Genotyping and imputation and QC.

DNA samples were genotyped on Affymetrix ArrayX arrays as previously described\textsuperscript{47}. Briefly, SNPs with allele frequency difference ≤0.15 between males and females for autosomal markers, and genotype concordance rate >0.75 across duplicate samples were included\textsuperscript{47}. Approximately 94% of samples and >98% of genetic markers assayed passed QC procedures. SNPs with genotype call rates <90% and SNPs with a MAF <1% were excluded. Following genotyping QC, we conducted statistical imputation of additional genetic variants. Following the pre-phasing of genotypes with ShapeIT v2.7.1958, variants were imputed from the cosmopolitan 1000 Genomes Project reference panel (phase I-integrated release; http://1000genomes.org) using IMPUTE2 v2.3.0. As a QC metric, we used the info\textsuperscript{2} statistic, which is an estimate of the correlation of the imputed genotype to the true genotype. Variants with an imputation\textsuperscript{2}<0.3 were excluded, and we restricted the imputation to SNPs that had a minor allele count ≥20 in the reference panel.

#### Defining genetic ancestry.

The genetic structure of GERA participants has been already characterized using genome-wide genotypes and PCs analysis\textsuperscript{23} and identified 10 and 6 ancestry PCs reflecting genetic ancestry among non-Hispanic whites, and the other ethnic groups, respectively\textsuperscript{21}. In this study\textsuperscript{47}, genetic ancestry PCs were calculated by Eigenstrat\textsuperscript{48} v4.2. In the current study, for genetic ancestry analyses, for each ethnic group, we focused on PC1 and PC2 because they are the only PCs that are geographically interpretable. In non-Hispanic whites, PC1 represents a European northwestern versus southeastern cline, and PC2 represents an African versus Native American ancestry. In Hispanic/Latinos, PC1 represents greater European versus Native American ancestry and PC2 represents greater African versus European ancestry.

#### Plots of cSCC prevalence versus genetic ancestry.

To visualize the cSCC prevalence distribution by the ancestry PCs, we created an ancestry contour figure based on a smoothed distribution of each individual’s cSCC phenotype (affected versus not) using a radial kernel density estimate weighted on the distance to each other individual, \( \sum_{i=1}^{n} \phi(|d_i|/\max|d_i|) \), where \( \phi() \) is the standard normal density distribution, \( k \) is the smoothing value (15 for non-Hispanic white
and Hispanic/Latino ethnic groups), and \(d(\cdot)\) is the Euclidean distance based on the first two PCs. A more detailed description of the method used to generate these plots has been published.\(^49\) Nationality subgroup labels were derived from the Human Genome Diversity Project for the visual representation of different subgroups from that sample.\(^21\) We have previously used these contour graphs to visualize genetic ancestry in other disorders in the GERA cohort.\(^66\) Ancestry contour figures using the viridis colors are also provided in the Supplementary Information.

HirisPlex-S program and predicted skin pigments. To determine whether the genetic ancestry associations with cSCC were due to differences in skin pigmentation, we predicted skin pigment traits (i.e., “dark skin,” “dark to black skin,” “intermediate skin,” “very pale skin,” and “pale skin”) using HirisPlex-S\(^24\)–\(^26\), a program that utilizes known pigment genetic variants to generate probabilities of different pigment traits. The HirisPlex-S predictive model includes 36 SNPs for skin color (Supplementary Table 3). We did consider using a five-category level (very pale, pale, intermediate, dark, and dark-black) of the HirisPlex-S skin pigmentation predictions and examined the distribution of all the categories in GERA non-Hispanic whites.

GRS based on cSCC-associated SNPs. To determine whether the known cSCC-associated loci could explain the observed associations of genetic ancestry with the risk of cSCC, we also included a weighted GRS in the logistic regression model. For SNP selection, we first investigated whether 16 SNPs associated with cSCC risk at a genome-wide significance level in previous GWAS studies\(^45\),\(^51\) were also associated with cSCC in GERA (Supplementary Data 2). SNPs rs192481803 and rs74899442 were not polymorphic (MAF < 1.0%) in any of the four GERA ethnic groups, and so we excluded at least one skin pigment probability. We chose to exclude “very pale”–“pale skin” from our models. As a result, the direction of the effect estimates of the skin pigment probabilities represents darker pigment.

GRS based on cSCC-associated SNPs. To determine whether the known cSCC-associated loci could explain the observed associations of genetic ancestry with the risk of cSCC, we also included a weighted GRS in the logistic regression model. For SNP selection, we first investigated whether 16 SNPs associated with cSCC risk at a genome-wide significance level in previous GWAS studies\(^45\),\(^51\) were also associated with cSCC in GERA (Supplementary Data 2). SNPs rs192481803 and rs74899442 were not polymorphic (MAF < 1.0%) in any of the four GERA ethnic groups, and so we excluded from the GRS and subsequent analyses. The 14 remaining SNPs (Supplementary Table 4) were confirmed to be associated with cSCC risk in GERA at Bonferroni significance (\(P < 0.05/14 = 0.0036\)) (Supplementary Data 2). The GRS was built on these 14 cSCC-SNPs by summing up the additive coding of each SNP weighted by the effect size ascertained from the original studies. As all previous GWAS of cSCC have been conducted in cohorts of European ancestry, we also generated unweighted GRS for the analyses conducted in the Hispanic/Latino sample. Results were similar using unweighted or weighted GRS in Hispanic/Latinos (Supplementary Table 11). Association of genetic ancestry with each skin pigmentation trait- and cSCC-associated SNP are presented in Supplementary Table 6.

Local ancestry. To determine whether the proportion of European ancestry at the 16 known cSCC- and/or skin pigmentation-associated loci could account for cSCC risk in Hispanic/Latinos, we conducted local ancestry analyses using LAMP-LD.\(^57\)

Fig. 3 Distribution of the five-category level of the HirisPlex-S skin pigmentation predictions in GERA non-Hispanic whites. Skin pigment traits (i.e., “dark skin,” “dark to black skin,” “intermediate skin,” “very pale skin,” and “pale skin”) were predicted using HirisPlex-S program based on 36 SNPs known to influence skin color. We did consider using a five-category level (very pale, pale, intermediate, dark, and dark-black) of the HirisPlex-S skin pigmentation predictions and examined the distribution of all the categories in GERA non-Hispanic whites.

We used the “HGDP-CEPH Human Genome Diversity Cell Line Panel”\(^52\) (https://hgap.org/hgdp/). Association testing between cSCC status and percent European ancestry at each locus was performed using the nonparametric test statistic proposed by Montana and Pritchard for admixture mapping.\(^53\) For this analysis, a Bonferroni-corrected significance threshold of 0.05/16 = 3.1 × 10^{-3} (accounting for the number of loci tested) was applied. We used the R package “ggsplot2” to generate the local ancestry at SLC24A5 plot presented in Fig. 2.

Variants prioritization. To identify which genetic variants within the 18 cSCC and/or skin pigmentation (14 cSCC + 4 conditional hits at known skin pigmentation loci) are likely to be causal, we used a Bayesian approach (CAVIARBF)\(^54\). For each of the 18 loci, we computed each variant’s capacity to explain the identified signal within a 2 Mb window (±1.0 Mb with respect to the original top variant) and derived the smallest set of variants that included the causal variant with 95% probability (95% credible set). A total of 7391 variants within 265 annotated genes were included in these 18 credible sets for the Hispanic/Latino analysis (Supplementary Data 5) and 1374 variants within 45 annotated genes were included in these 18 credible sets for the non-Hispanic white analysis (Supplementary Data 6).

Statistical analysis. We used a logistic regression model to examine the impact of ancestry on cSCC risk using R version 3.4.1 with the following covariates: age, sex, and ancestry PCs (Model 1). For all the analyses, we included as covariates the top 10 ancestry PCs for the non-Hispanic whites, whereas we included the top 6 ancestry PCs for the Hispanic/Latinos. To adjust for genetic ancestry, we also included the percentage of Ashkenazi (ASHK) Jewish ancestry as a covariate for the non-Hispanic white ethnic group analysis. We present the full multivariate logistic regression models of cSCC and ncSCC in Supplementary Tables 1, 2, 6, and 8, including all the PCs for each ethnic group, and adjusting for age and sex. In Model 1, in addition to all covariates included in Model 1, we added skin pigment probabilities from HirisPlex-S. In Model 3, in addition to all covariates included in Model 2, we added the GRS described above. In Model 4, in addition to all covariates included in Model 3, we added history of AK as a covariate, reflecting sun exposure. The proportion of variance explained by each covariate (i.e., genetic ancestry PC, HirisPlex-S skin pigment probabilities, GRS based on 14 known cSCC-associated SNPs, or history of AK) and for each ethnic group (i.e., non-Hispanic white or Hispanic/Latino) are reported in Table 3. For the conditional analysis of the skin pigmentation loci for cSCC association, we incorporated 14 previously reported cSCC risk SNPs as covariates.

Analyses of ncSCC. To assess whether ncSCC followed a similar pattern of genetic ancestry associations to cSCC, we conducted a parallel analysis of ncSCC in GERA, evaluating the association of genetic ancestry and potential modification by skin pigment and previously reported cSCC-SNPs.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Genotype data of GERA participants are available from the database of Genotypes and Phenotypes (dbGaP) under accession phs000674.v2.p2. This includes individuals who consented to having their data shared with dbGaP. The complete GERA data are available upon application to the KP Research Bank (https://researchbank.kaiserpermanente.org/).

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References

1. Loh, T. Y., Ortiz, A., Goldenberg, A. & Brian Jiang, S. I. Prevalence and clinical characteristics of nonmelanoma skin cancers among hispanic and asian patients compared with white patients in the united states: a 5-year, single-institution retrospective review. Dermatol. Surg. 42, 639–645 (2016).
2. Karia, P. S., Han, J. & Schmults, C. D. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. J. Am. Acad. Dermat. 68, 957–966 (2013).
3. Rogers, H. W., Weinstock, M. A., Feldman, S. R. & Coldiron, B. M. Incidence estimate of nonmelanoma skin cancer (keratinocyte carcinomas) in the U.S. population, 2012. JAMA Dermat. 151, 1081–1086 (2015).
4. Rogers, H. W. et al. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. Arch. Dermat. 146, 283–287 (2010).
5. Muzic, J. G. et al. Incidence and trends of basal cell carcinoma and cutaneous squamous cell carcinoma: a population-based study in Olmsted County, Minnesota, 2000 to 2010. Mayo Clin. Proc. 92, 890–898 (2017).
6. Jackson, B. A. Nonmelanoma skin cancer in persons of color. Semin. Cutan. Med. Surg. 28, 93–95 (2009).

7. Stang, A. Genital and nongenital nonmelanoma skin cancer: more epidemiological studies are needed. J. Invest. Dermatol. 127, 2296–2299 (2007).

8. Kricker, A., Armstrong, B. K., English, D. R. & Heenan, P. J. Pigmentary and cutaneous risk factors for non-melanocytic skin cancer—a case-control study. Int. J. Cancer 48, 650–662 (1991).

9. Green, A., Battistutta, D., Hart, V., Leslie, D. & W edden, D. Skin cancer in a subtropical Australian population: incidence and lack of association with occupation. The Nambour Study Group. Am. J. Epidemiol. 144, 1034–1040 (1996).

10. Moan, J., Grigalavicus, M., Baturaxe, Z., Juuseniemi, A. & Dahlback, A. North-South gradients of melanomas and non-melanomas: A role of vitamin D? Dermatologicaendocrinology 5, 186–191 (2013).

11. Mucci, L. A. et al. Familial risk and heritability of cancer among twins in Nordic countries. JAMA 315, 68–76 (2016).

12. Sordillo, J. E., Kraft, P., Wu, A. C. & Asgari, M. M. Quantifying the polygenic contribution to cutaneous squamous cell carcinoma risk. J. Invest. Dermatol. 138, 1307–1310 (2018).

13. Stacey, S. N. et al. New common variants affecting susceptibility to basal cell carcinoma. Nat. Genet. 41, 909–914 (2009).

14. Han, J. et al. A germline variant in the interferon regulatory factor 4 gene as a novel skin cancer risk locus. Cancer Res. 71, 1533–1539 (2011).

15. Nick, H., Kraft, P., Hunter, D. J. & Han, J. Genetic variants in pigmentation genes, pigmentedary phenotypes, and risk of skin cancer in Caucasians. Int. J. Cancer 125, 909–917 (2009).

16. Yoshizawa, J. et al. Variants in melanogenesis-related genes associate with skin cancer risk among Japanese populations. J. Dermatol. 41, 296–302 (2014).

17. Auger, M. M., Toland, A. E. & Arron, S. T. IRF4 polymorphism is associated with cutaneous squamous cell carcinoma in organ transplant recipients: a pigment-independent phenomenon. J. Invest. Dermatol. 137, 251–253 (2017).

18. Asgari, M. M. et al. Identification of susceptibility loci for cutaneous squamous cell carcinoma. J. Invest. Dermatol. 136, 930–937 (2016).

19. Chahal, H. S. et al. Genome-wide association study identifies novel susceptibility loci for cutaneous squamous cell carcinoma. Nat. Commun. 7, 12048 (2016).

20. Elhaik, E. et al. Geographic population structure analysis of worldwide human populations infers their biogeographical origins. Nat. Commun. 5, 3513 (2014).
