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ASIP genetic variants and the number of non-melanoma skin cancers

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Abstract

Patients with primary non-melanoma skin cancers (NMSCs) tend to develop these cancers at multiple independent sites. We examined the genetic factors in the development of multiple NMSCs among Caucasian women with 28 years of follow-up. We initially evaluated 19 SNPs in nine pigmentation genes with the number of NMSCs in 492 cases and 619 controls without a

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history of NMSC. We found nominal significant associations between two *ASIP* gene–related SNPs, rs1885120 and rs910873, and an *ASIP* haplotype (AH) (rs4911414 allele T and rs1015362 allele G) and an increased number of NMSCs, with *p*-values of 0.008, 0.01, and 0.01, respectively. We further evaluated these two SNPs and AH haplotype in three data sets. In a joint analysis with 1,507 cases and 4,335 controls, AH haplotype was independently associated with the number of NMSCs with odds ratio (OR) (95% confidence interval (CI)) of 1.45(1.25–1.68) (*p*-value = 6.2E–07). The AH haplotype was associated with an increased risk of developing one NMSC (OR 1.32; 95% CI, 1.07–1.63). The OR increased to 1.45(1.18–1.78) for those with 2–4 NMSCs and 1.84(1.34–2.53) for those with at least five. The findings suggest that *ASIP* locus is associated with the number of NMSCs.

Keywords

SNP; Pigmentation gene; Non-melanoma skin cancer; ASIP

Introduction

Non-melanoma skin cancers (NMSCs) are the most common cancer in the white population. There are approximately one million cases each year in the United States, which accounts for half of all cancers in the country [1]. The incidence of non-melanoma skin cancer in the United States almost doubled from 1992 through 2006 [2]. Patients with primary NMSCs tend to develop these cancers at multiple independent sites; the 3-year cumulative risk of developing additional NMSC after the first diagnosis ranges from 35 to 60% with a mean risk of 47% [3]. The risk factors for multiple NMSCs are not well defined, but studies have suggested that the type and the number of prior NMSC are highly associated with subsequent risk [3]. Data on genetic factors that may contribute to the development of multiple NMSCs have been limited. Some studies previously reported the associations between carcinogen-metabolizing enzymes and the number of NMSCs [4–8], including glutathione S-transferase (GSTM1, GSTT1, GSTM3, and GSTP1) and cytochrome P450 (CYP2D6 and CYP1A1) [4–8].

Lighter pigmentation is a well-known phenotypic risk factor for skin cancer [9]. Recent studies have reported the associations between some pigmentation genes and increased risks of melanoma and NMSC [10–14]. In the present study, we evaluated the association between 19 single-nucleotide polymorphisms (SNPs) in nine candidate pigmentation genes (*TYR, TYRP1, OCA2, SLC24A5, SLC24A4, SLC45A2, POMC, ASIP*, and *ATRN*) and the number of NMSCs a person develops, and further replicated the findings on the *ASIP* gene.

Materials and methods

Study design overview

This study was conducted within the Nurses' Health Study (NHS) in two stages. First, we evaluated the association between the 19 SNPs and the risk of NMSCs in the skin cancer nested case–control study as the discovery set. We further examined the four *ASIP* SNPs in three independent data sets, in which we had directly genotyped or imputed data on these four SNPs.

Study cohort

The NHS was established in 1976, when 121,700 female registered nurses between the ages of 30 and 55 residing in 11 larger US states completed and returned the initial self-administered questionnaire on their medical histories and baseline health-related exposures,

forming the basis for the NHS cohort. Updated exposure information and disease status have been obtained by questionnaires every 2 years. Between 1989 and 1990, blood samples were collected from 32,826 volunteers of the cohort members. We restricted our study to Caucasians.

Non-melanoma skin cancer includes squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). All medical records of SCC cases were reviewed by dermatologists blinded to exposure information according to established criteria. The report was classified as confirmed cancer only if confirmed by the pathology report. We do not confirm self-reported BCC diagnosis in our cohort. Our previous validation study showed 90% pathology confirmation rate of self-reports of a single BCC diagnosis in a subset of our cohort [15,16]. In addition, we observed strong associations between well-known skin cancer host risk factors and exposures and BCC risk in our cohort [9], which further reassures that the self-report of BCC holds validity in our cohort.

Outcome ascertainment

Information on the cumulative number of NMSCs was collected through the 2004 cohort follow-up questionnaire. The question was, “How many squamous or basal cell carcinoma lesions have you ever had removed by surgery, cryotherapy or other means? (Include only new primary cancers. Exclude melanoma and benign lesions like moles or actinic keratoses).” The possible choices were never, 1, 2–4, 5–10, and 11+. To minimize the misclassification of the outcome, the eligible cases were Caucasians who reported at least one NMSC in this question and had pathologically confirmed diagnosis of SCC or self-reported BCC in the cohort follow-up from 1976 up to 2004. According to the number of NMSCs removed, cases were further categorized as cases [1], cases [2–4], and cases (5+). The eligible controls were Caucasians who answered “never” to this question and did not report any skin cancer diagnosis in the cohort biennial questionnaires up to 2004.

Exposure data

We obtained information regarding skin cancer risk factors from the prospective biennial questionnaires. Information on natural hair color at age 20 as well as childhood and adolescent tanning ability and burning tendency was collected in the 1982 prospective questionnaire. The variables were classified into five categories for hair color (black, dark brown, light brown, blonde, and red), four categories for tanning ability (practically none, light tan, average tan, and deep tan), and four categories for burning tendency (practically none, some redness only, burn, and painful burn with blisters).

Study population for discovery phase

The discovery set consisted of 803 Caucasian skin cancer cases and 870 age-matched Caucasian controls from the subcohort who gave a blood specimen within the NHS. There were 285 pathologically confirmed SCC cases and 300 self-reported BCC cases randomly selected from ~2,600 available self-reported BCC cases. Melanoma cases (n = 218) were excluded from this study. Detailed description on this study was published previously [17].

Study populations for replication phase

Eligible subjects in the replication set consisted of women in the three existing nested case–control studies from the subcohort who gave a blood specimen within the NHS: postmenopausal invasive breast cancer (replication set 1), the type 2 diabetes (replication set 2), and coronary heart disease (replication set 3). Detailed description on these three studies was published previously [18–20]. In these three studies, we excluded those who had

pathologically confirmed diagnosis of melanoma up to 2004. There was no sample overlap between the discovery set and the three replication sets.

Genotyping data in the discovery set

We chose the putative functional SNPs in nine pigmentation genes, including non-synonymous SNPs, and those in the promoter and UTR regions, and those identified from recent genome-wide association studies (GWASs) on pigmentary phenotypes and skin cancer. Some of these SNPs have been found to be associated with pigmentary phenotypes, including fair skin color, hair color, and tanning tendency; others are also risk factors for either melanoma or NMSC [10,11,14,21–24].

In the discovery set, we genotyped all the 19 SNPs except for rs910873 and rs1885120 in nine candidate pigmentation genes (*TYR*, *TYRP1*, *OCA2*, *SLC24A5*, *SLC24A4*, *SLC45A2*, *POMC*, *ASIP*, and *ATRN*) using the OpenArray™ SNP Genotyping System (Applied Biosystems, Foster City, CA). We used the five nuclease assay (TaqMan®) in the 384-well format to genotype rs910873 and rs1885120 using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Due to the assay failure, we genotyped rs1393350 as a surrogate for the *TYR* Arg402Gln (rs1126809) ($D' = 1$ and $ra^2 = 0.86$) (http://snp500cancer.nci.nih.gov). TaqMan® primers and probes were designed using the Primer Express® Oligo Design software v2.0 (ABI PRISM). Laboratory personnel were blinded to case–control status, and 42 blinded quality control samples were inserted to validate genotyping procedures; concordance for the blinded samples was 100%. The call rates for all 19 SNPs were >95%. Primers, probes, and conditions for genotyping assays are available upon request.

Genotyping and imputation in the replication sets

We performed genotyping in the three replication sets within the NHS using either the Illumina 550 or Affymetrix 6.0 SNP chip. The four *ASIP* SNPs were either directly genotyped or imputed with high imputation quality (R square, 0.94–0.99) based on the genotyped SNPs and haplotype information in the Hapmap phase II data build 35 (CEU) using the computationally efficient hidden Markov model implemented in MACH [17] (Supplementary Table 3). We used the STRUCTURE program to determine whether the subjects had an admixed origin with either Asian or African ancestry. Samples with evidence of intercontinental admixture were excluded [19,20,25]. Controlling for case–control status in the replications sets (breast cancer, type 2 diabetes, and coronary heart disease) made no material difference to the results on the pigmentation SNPs in relation to the number of NMSC.

Statistical methods

We carried out association analysis in the discovery set and each of the replication sets. We then carried out a joint analysis combing the discovery set and the three replication sets. We used the χ^2 test to assess whether the genotypes for all 19 SNPs were in Hardy–Weinberg equilibrium among the controls in the discovery set.

We used unconditional logistic regression to evaluate the association between each SNP and the number of NMSCs. The genotype was coded ordinally (0, 1, or 2 copies of SNP minor allele) in additive models. In the discovery set and replication sets, ordinal logistic regression models were used to provide the *p*-value for cumulative odds ratio (OR) of each SNP in relation to the risk of multiple NMSCs. In the joint analysis, multinomial logistic regression models were used to calculate the OR for each of the three outcome groups (1, 2-4, and 5+) compared to “none.” Tanning tendency was not significantly associated with

the risk of multiple NMSCs after adjusting for other risk factors, and we did not include it in the multivariate logistic regression models.

In the haplotype analyses, a simple expectation–maximization algorithm was used to estimate haplotype frequencies and expected counts of haplotypes for individuals [26]. Expectation substitution was used for association analyses of haplotype and the risk of multiple NMSCs. All statistical analyses were two sided and carried out using SAS V9.1 (SAS Institute, Cary, NC).

We carried out a sensitivity analysis adjusting for the three largest principal components of genetic variation in the regression model in the joint analysis. These principal components were calculated for all individuals on the basis of ca. 10,000 unlinked markers using the EIGENSTRAT software [27]. The top three eigenvectors were chosen on the basis of significant ($p < 0.05$) Tracy-Wisdom tests [28]. Adjusting for up to the top 50 principal components did not further reduce the genomic control inflation factor.

Results

This study included 1,507 cases of single or multiple NMSCs and 4,335 controls. Among the cases, 679 participants reported only one NMSC, 622 reported two to four NMSCs, while 206 reported at least five NMSCs. Compared with controls, the cases were more likely to have red or blonde hair color. Cases with only one NMSC had distribution of tanning ability and burning tendency similar to those of the controls, whereas cases with more than one NMSC tended to have less tanning ability and more likely to burn after sun exposure (Table 1). Overall, the missing data on these covariates is minimal (4.6% for hair color, 4.4% for tanning ability, and 5.5% for burning tendency).

Information on the 19 SNPs is shown in Supplementary Table 1. The distributions of the genotypes for these SNPs were in Hardy–Weinberg equilibrium among controls. We first examined the association between each SNP and the number of NMSCs in the discovery set, which included 492 NMSC cases and 619 selected controls (Supplementary Table 2). We observed that two *ASIP*-related SNPs, rs1885120 and rs910873, were nominally significantly associated with an increased number of NMSCs with age-adjusted OR (95% confidence interval (95%CI)) of 1.46 (1.10–1.94) (*p*-value = 0.008) and 1.43 (1.09–1.89) (*p*-value = 0.01), respectively.

The *ASIP* AH haplotype carrying rs4911414 variant allele T and rs1015362 major allele G was reported previously to be responsible for the signal in the *ASIP* region in relation to pigmentary phenotypes [23] and was associated with the risk of BCC [13]. In the discovery set, we found that the AH haplotype was associated with the number of NMSCs. The age-adjusted OR (95% CI) was 1.45 (1.08–1.93) with *p*-value of 0.01. The *ASIP* AH haplotype was in high linkage disequilibrium (LD) with the two SNPs (rs910873 and rs1885120) (R square = 0.7). The two SNPs rs910873 and rs1885120 were in strong LD (R square >0.9). The *ASIP* SNP rs6058017 was not included in the Hapmap release 22 project and hence was not imputed in our replication datasets. The details on genotype and imputation of the four *ASIP* SNPs are listed in Supplementary Table 3.

In the breast cancer set (replication set 1) with 370 cases and 1,291 controls, rs910873 and AH haplotype were nominally significantly associated with multiple NMSCs with *p*-values of 0.02 and 0.009, respectively (rs1885120, $p = 0.07$). In the type 2 diabetes set (replication set 2) with 479 cases and 1,828 controls, the *p*-values of rs910873, rs1885120, and AH haplotype were 3.3E–04, 4.5E–04, and 0.003, respectively (Table 2). In the coronary heart disease set (replication set 3) with 166 cases and 597 controls, the associations were in the

same direction but not statistically significant, probably due to a relatively small sample size in this set or chance.

We further carried out a pooled analysis with 1,507 NMSC cases and 4,335 controls combining the four datasets. The age-adjusted model showed significant associations between the rs910873, rs1885120, and AH haplotype and the risk of multiple NMSCs (Table 2). The ORs (95%CI) were 1.35 (1.20–1.53) (p = 8.4E–07), 1.34 (1.18–1.51) (p = 3.2E–06), and 1.45(1.25–1.68) (p = 6.2E–07), respectively. The risk estimate remained similar after additionally adjusting for the top three principal components of genetic variance. The OR (95%CI) was 1.39 (1.18–1.64) for rs910873, 1.36 (1.15–1.61) for rs1885120, and 1.41 (1.18–1.67) for the AH haplotype.

We further assessed the risk estimate of these variants for each case group (Table 3). The magnitude of risk increased as more NMSCs developed for rs910873 and rs1885120 and for the AH haplotype. Compared with controls, among cases with only one NMSC, the AH haplotype was associated with an increased risk with OR (95% CI) of 1.32(1.07–1.63). The OR increased to 1.45 (1.18–1.78) for those who had 2–4 NMSCs, and 1.84 (1.34–2.53) for those with at least five NMSCs. In models with mutual adjustment for rs910873 (or rs1885120) and AH haplotype, the association of rs1885120 (or rs910873) was eliminated and became non-significant, while the association of AH haplotype remained significant.

To test whether pigmentary phenotypes serve as the intermediate along the pathway between the SNPs and the risk of multiple NMSCs, we additionally adjusted for burning tendency and hair color. The associations of the two SNPs and AH haplotype were modestly attenuated but remained significant (Table 3).

Discussion

Agouti signaling protein, encoded by the *ASIP* gene, binds to melanocortin 1 receptor (MC1R) as an antagonist to α-melanocyte-stimulating hormone (α-MSH) and blocks the MC1R-stimulated elevation of cAMP, resulting in pheomelanogenesis. It has been reported that the *ASIP* gene is not only related to pigmentation traits but is also involved in the etiology of skin cancer. A recent GWAS identified rs910873 and rs1885120 as melanoma skin loci [10]. The rs910873 and rs1885120 are 315 and 720 kb downstream from the *ASIP* gene, respectively. Studies by our group suggested that these polymorphisms were associated with light pigmentary phenotypes and increased risks of BCC and SCC [29]. Because rs910873 and rs1885120 are highly correlated ($r^2 > 0.9$), we found a similar magnitude of association between each of the two SNPs and the number of NMSCs. In addition, the AH haplotype at the *ASIP* locus carrying rs4911414 variant allele T and rs1015362 major allele G was also significantly associated with pigmentary traits as well as with increased risks of melanoma, BCC, and SCC [13,14,23,29]. In the present study, we also found that the AH haplotype was associated with the number of NMSCs.

We found that the association of rs910873 (or rs1885120) was eliminated in the presence of the AH haplotype, while that of the AH haplotype remained significant. This suggests that the AH haplotype is a stronger locus than rs910873 or rs1885120 for the number of NMSCs. The haplotype is a particularly strong risk factor for developing five or more NMSCs, as demonstrated in a monotonic relationship between AH haplotype–associated risk and the number of NMSCs. This suggests that the AH haplotype was not only associated with the incidence of NMSC, but also associated with an increased risk of developing multiple NMSCs. In addition, we observed that the haplotype ASIP AH was significantly associated with fair skin color (OR, 2.28; 95% CI, 1.46–3.57) (p-value for global test, 0.003) [14]. In this study, we found that the associations with the number of NMSC were attenuated after

adjusting for pigmentary phenotypes, which suggested that these associations were at least partially mediated through the pigmentation pathway. The ASIP regulates MC1R-related pigmentation pathway, and it is plausible that the AH haplotype may affect the *ASIP* gene expression, influence the pigmentation production, and in turn confer the susceptibility to non-melanoma skin cancer. To strengthen this association, we observed in this study that the magnitude of risk increased as more NMSCs developed for the AH haplotype.

There are several limitations of our study. Participants were not a random sample of US women. However, it seems unlikely that the basic biologic relations among participants in the NHS will differ from those of the general population. There is a potential for misclassification of the outcome. To minimize this, we defined the eligible cases as those who reported at least one NMSC in the question on the number of NMSCs in 2004 and had pathologically confirmed diagnosis of SCC or self-reported BCC in the cohort follow-up from 1976 up to 2004, and the eligible controls as those who answered 'never' to this question and did not report any skin cancer diagnosis in the cohort biennial questionnaires up to 2004. Because of the way we collected the information on the number of NMSCs retrospectively, we would not be able to differentiate those cases with multiple NMSCs diagnosed sequentially and those diagnosed at the same time. In addition, we do not have sufficient information to identify the two types of NMSC. The majority of the cases in our study are BCC, which has a much higher incidence rate and tendency for relapsing than SCC [3]. Our analysis excluding those with SCC diagnosis yielded similar results. In addition, light pigmentation is a well-known common risk factor for both SCC and BCC, which suggests that the pigmentation pathway may be one common underlying mechanism for developing non-melanoma skin cancer. Modest sample size in the discovery set limited our power to detect additional signals. We only evaluated the associations among women. Because of different sun exposure patterns, it is important to confirm these findings in men.

In summary, we identified that the *ASIP* genetic variants were associated with the number of NMSCs in Caucasian women. This association is likely to be driven by the *ASIP* AH haplotype. Our results suggest that *ASIP* AH haplotype is associated with an increased risk of developing multiple NMSCs, which may be informative to identify those Caucasian women who tend to have more than one NMSC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1
Characteristics of cases and controls within the Nurses' Health Study (1976–2004)

Characteristics	Controls	Cases [1]	Cases [2–4]	Cases (5+)
Discovery set (skin cancer)	619	187	223	82
Replication 1 (breast cancer)	1291	168	153	49
Replication 2 (type 2 diabetes)	1828	229	192	58
Replication 3 (coronary heart disease)	597	95	54	17
Age at 2004 (years)				
Discovery set	72.1	73.0	72.7	72.3
Replication 1	71.5	73.5	74.2	74.3
Replication 2	70.1	72.1	73.1	74.8
Replication 3	72.7	75.0	73.6	76.6
BMI at 2004 (kg/m2)				
Discovery set	25.9	25.5	26.0	25.6
Replication 1	26.3	25.1	25.5	24.0
Replication 2	28.1	27.6	26.7	27.0
Replication 3	25.8	24.9	24.6	27.6
Natural hair color at age 20 red or blonde (%)				
Discovery set	14	18	20	27
Replication 1	14	13	25	31
Replication 2	14	21	20	24
Replication 3	14	23	13	35
Tanning ability (average and deep tan) (%)				
Discovery set	66	67	54	51
Replication 1	70	67	54	53
Replication 2	65	63	54	50
Replication 3	64	68	59	47
Burning tendency (burn and painful burn with blisters) (%)				
Discovery set	35	37	48	65
Replication 1	31	33	45	53
Replication 2	33	43	54	61
Replication 3	34	38	37	71

Table 2

Associations between the ASIP-selected SNPs and haplotypes and the risk of different numbers of non-melanoma skin cancers

SNP	Discovery set		Replication 1		Replication 2		Replication 3		Combined	
	OR (95% CI)[a]	*p*-value	OR (95% CI)[a]	*p*-value	OR (95% CI)[a]	*p*-value	OR (95% CI)[a]	*p*-value	OR (95% CI)[a]	*p*-value
rs910873	1.43 (1.09–1.89)	0.01	1.39 (1.05–1.83)	0.02	1.52 (1.21–1.92)	3.3E–04	1.11 (0.71–1.74)	0.63	1.35 (1.20–1.53)	8.4E–07
rs1885120	1.46 (1.10–1.94)	0.008	1.30 (0.98–1.71)	0.07	1.52 (1.20–1.92)	4.5E–04	1.16 (0.74–1.81)	0.53	1.34 (1.18–1.51)	3.2E–06
AH haplotype[b]	1.45 (1.08–1.93)	0.01	1.47 (1.11–1.96)	0.009	1.47 (1.15–1.88)	0.003	1.17 (0.74–1.87)	0.32	1.45 (1.25–1.68)	6.2E–07

[a] Age-adjusted ordinal logistic regression model with cumulative OR and *p*-value

[b] ASIP AH carrying rs4911414 variant allele T and rs1015362 major allele G

Table 3

Association between three selected SNPs and the risk of different numbers of non-melanoma skin cancers in the joint analysis

	Controls	Cases[1][b]	Cases[2–4][b]	Cases(5+)[b]	Cumulative OR	*p*-value[c]
rs910873						
Age-adjusted OR (95% CI)	1(Ref)	1.26 (1.03–1.53)	1.49 (1.23–1.81)	1.64 (1.20–2.22)	1.35 (1.20–1.53)	8.4E–07
Multivariate OR (95% CI)[a]	1(Ref)	1.20 (0.98–1.46)	1.32 (1.08–1.62)	1.39 (1.01–1.91)	1.25 (1.11–1.42)	0.0004
rs1885120						
Age-adjusted OR (95% CI)	1(Ref)	1.24 (1.01–1.51)	1.47 (1.21–1.80)	1.60 (1.17–2.19)	1.34 (1.18–1.51)	3.2E–06
Multivariate OR (95% CI)[a]	1(Ref)	1.18 (0.96–1.45)	1.29 (1.04–1.58)	1.37 (0.99–1.90)	1.24 (1.09–1.44)	0.001
AH haplotype						
Age-adjusted OR (95% CI)	1(Ref)	1.32 (1.07–1.63)	1.45 (1.18–1.78)	1.84 (1.34–2.53)	1.45 (1.25–1.68)	6.2E–07
Multivariate OR (95% CI)[a]	1(Ref)	1.25 (1.01–1.55)	1.25 (1.00–1.56)	1.52 (1.09–2.11)	1.28 (1.10–1.49)	0.002

[a] Adjusted for age and hair color and burning tendency

[b] Multinominal logistic regression

[c] The *p*-value corresponds to the cumulative OR