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Genetic variants in pigmentation genes, pigmentary phenotypes, and risk of skin cancer in Caucasians

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Abstract

Human pigmentation is a polygenic quantitative trait with high heritability. Although a large number of single nucleotide polymorphisms (SNPs) have been identified in pigmentation genes, very few SNPs have been examined in relation to human pigmentary phenotypes and skin cancer risk. We evaluated the associations between fifteen SNPs in eight candidate pigmentation genes (*TYR, TYRP1, OCA2, SLC24A5, SLC45A2, POMC, ASIP*, and *ATRN*) and both pigmentary phenotypes (hair color, skin color, and tanning ability) and skin cancer risk in a nested case-control study of Caucasians within the Nurses' Health Study (NHS) among 218 melanoma cases, 285 squamous cell carcinoma (SCC) cases, 300 basal cell carcinoma (BCC) cases, and 870 common controls. We found that the *TYR* Arg402Gln variant was significantly associated with skin color (p-value $=7.7\times10^{-4}$) and tanning ability (p-value $=7.3\times10^{-4}$); the *SLC45A2* Phe374Leu variant was significantly associated with hair color (black to blonde) (p-value $=2.4\times10^{-7}$), skin color (p-value $=1.1\times10^{-7}$), and tanning ability (p-value $=2.5\times10^{-4}$). These associations remained significant after controlling for *MC1R* variants. No significant associations were found between these polymorphisms and the risk of skin cancer. We observed that the *TYRP1* rs1408799 and *SLC45A2* -1721 C>G were associated with melanoma risk (OR, 0.77; 95% CI, 0.60–0.98 and OR, 0.75; 95% CI, 0.60–0.95, respectively). The *TYR* Ser192Tyr was associated with SCC risk (OR, 1.23; 95% CI, 1.00–1.50). The *TYR* haplotype carrying only the Arg402Gln variant allele was significantly associated with SCC risk (OR, 1.35; 95% CI, 1.04–1.74). The *OCA2* Arg419Gln and *ASIP* g.8818 A>G were associated with BCC risk (OR, 1.50; 95% CI, 1.06–2.13 and OR, 0.73; 95% CI, 0.53–1.00, respectively). The haplotype near *ASIP* (rs4911414[T] and rs1015362[G]) was significantly associated with fair skin color (OR, 2.28; 95% CI, 1.46–3.57) as well as the risks of melanoma (OR, 1.68; 95% CI, 1.18–2.39) and SCC (OR, 1.54; 95% CI, 1.08–2.19). These associations remained similar after adjusting for pigmentary phenotypes and *MC1R* variants. The statistical power of this study was modest and additional studies are warranted to confirm the associations observed in the present study. This study provides evidence for the contribution of pigmentation genetic variants, in addition to the *MC1R* variants, to variation in human pigmentary phenotypes and possibly the development of skin cancer.

Keywords

SNPs; pigmentation gene; pigmentary phenotypes; skin cancer

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Introduction

Human pigmentation shows substantial variation both within and among human populations, with high heritability [1, 2]. Ultraviolet (UV) exposure is one of the most important environmental variables partially influencing evolutionary selective pressure on human pigmentation [3]. Melanin synthesized within melanosomes in melanocyte is the main contributor to human pigmentation. There are two main types of melanin: pheomelanin (red or yellow) and eumelanin (black or brown) [4].

It has been hypothesized that human pigmentation is tightly regulated by multiple pigmentation genes harboring a handful of genetic variants (Figure 1). The genes involved in the process of pigmentation, such as formation, transport, and distribution of melanosome, have been identified through animal models [5]. Melanin production is initiated by α-melanocyte-stimulating hormone (α-MSH), which is produced by proteolysis from a multicomponent precursor polypeptide encoded by the pro-opiomelanocortin (*POMC*) gene [6]. A previously well-documented pigmentation gene, *MC1R* (melanocortin 1 receptor), encodes a 317-amino acid 7-pass transmembrane G-protein coupled receptor. As an agonist of *MC1R*, the induced POMC/α-MSH binds to *MC1R*, leading to elevated cAMP levels and resulting in eumelanin production [7, 8]. Alternatively, the agouti signaling protein (*ASIP*) can also bind to the *MC1R*, blocking the *MC1R*-stimulated elevation of cAMP, and over-expression of *ASIP* produces a yellow coat color in mice [5, 9, 10]. Attractin encoded by the *ATRN* gene is a low-affinity receptor for the ASIP protein product. A recessive color mutation mahogany ($Atrn^{mg}$) was recognized as a modifier of agouti coat color in mice [11, 12]. Tyrosinase (*TYR*) is required for melanization in both types of melanosome, whereas the tyrosinase-related protein 1 (*TYRP1*) is exclusive to the melanization of eumelanosome [13, 14]. Hence, tyrosinase is a critical enzyme during melanosomal maturation and its high activity leads to the formation of eumelanosome [15, 16]. The optimal activity of tyrosinase in human melanocytes requires an appropriate ionic environment, which is partially controlled by P-protein functioning as a pH exchange membrane channel [17, 18]. The twelve transmembrane-spanning P-protein encoded by the *OCA2* gene (human type II oculocutaneous albinism-related gene) was discovered in the “pink-eyed dilution” mouse mutant [19]. In addition to P-protein, MATP, a membrane-associated transporter protein encoded by the *SLC45A2* gene, has been considered as a sodium-hydrogen exchanger of melanosomes, regulating tyrosinase activity in human melanocyte [20]. Another cation exchanger, *SLC24A5*, transports calcium or potassium ions into the melanosome and is involved in melanogenesis. It has been proposed that the human *SLC24A5* gene is required for maturation of melanosome and has a role in skin pigmentation [21, 22].

Lighter pigmentation is the host susceptibility risk factor for skin cancer [23]. Although a large number of single nucleotide polymorphisms (SNPs) were identified in pigmentation genes, very few SNPs have been examined in relation to human pigmentary phenotypes and skin cancer risk. Recent genome-wide association studies on pigmentary traits and skin cancer risks (melanoma and basal cell carcinoma) have generated additional information on both pigmentary phenotype related- and skin cancer related-genetic variants [24–28]. In the present study, we evaluated the associations of fifteen SNPs in eight candidate pigmentation genes (*TYR*, *TYRP1*, *OCA2*, *SLC24A5*, *SLC45A2*, *POMC*, *ASIP*, and *ATRN*) with both pigmentary phenotypes (hair color, skin color, and tanning ability) and the risk of melanoma and non-melanoma skin cancer (squamous cell carcinoma (SCC) and basal cell carcinoma (BCC)) in a nested case-control study within the Nurses’ Health Study (NHS).

Materials and Methods

Study population

The NHS was established in 1976, when 121,700 female registered nurses between the ages of 30 and 55, residing in 11 larger U.S. 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 information has been obtained by questionnaires every two years. From May 1989 through September 1990, we collected blood samples from 32,826 participants in the NHS cohort. The distributions of risk factors for skin cancer were very similar in the subcohort of those who donated blood samples as in the overall cohort [29]. Eligible cases in this study consisted of women with incident skin cancer from the subcohort who had given a blood specimen, including SCC and BCC cases with a diagnosis anytime after blood collection up to June 1, 1998 and melanoma cases up to June 1, 2000 that had no previously diagnosed skin cancer. All available pathologically confirmed melanoma and SCC cases and 300 self-reported BCC cases randomly selected from ~2,600 available self-reported BCC cases were included. The validity of self-report of BCC is high in this medically sophisticated population (90%) [30, 31]. A common control series was randomly selected from participants who gave a blood sample and were free of diagnosed skin cancer up to and including the questionnaire cycle in which the case was diagnosed. One or two controls were matched to each case by year of birth (±1 year). All subjects were the U.S. non-Hispanic Caucasian women in this study. The nested case-control study consisted of 218 melanoma cases, 285 SCC cases, a sample of 300 BCC cases from the large number of incident cases, and 870 matched controls. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women's Hospital, Boston, MA.

Exposure data

We obtained information regarding skin cancer risk factors from the prospective biennial questionnaires and a retrospective supplementary questionnaire. Information on natural hair color at age 20 and childhood and adolescent tanning tendency were collected in the 1982 prospective questionnaire into five categories (black, dark brown, light brown, blonde, and red) and four categories (practically none, light tan, average tan, and deep tan), respectively. Question on ethnic group was ascertained in the 1992 questionnaire. In the skin cancer nested case-control study, natural skin color and other sun exposure-related information were collected by the retrospective supplementary questionnaire in 2002. The response rate of cases and controls were 92% and 89%, respectively. Information on natural skin color was classified into three categories (fair, medium, and olive). In addition, the eleven states of residence of cohort members at baseline were grouped into three regions: Northeast (Connecticut, Massachusetts, Maryland, New Jersey, New York, and Pennsylvania), Northcentral (Michigan and Ohio), and West and South (California, Texas, and Florida).

Laboratory assays

We genotyped fifteen SNPs in eight candidate pigmentation genes (*TYR*, *TYRP1*, *OCA2*, *SLC24A5*, *SLC45A2*, *POMC*, *ASIP*, and *ATRN*) using the OpenArray™ SNP Genotyping System (BioTrove, Woburn, MA). Due to the assay failed we genotyped rs1393350 as a surrogate for the *TYR* Arg402Gln (rs1126809) ($D'=1$ and $r^2=0.86$) (http://snp500cancer.nci.nih.gov). Laboratory personnel were blinded to the case-control status, and 42 blinded quality control samples were inserted to validate genotyping procedures; concordance for the blinded samples was 100%. Primers, probes, and conditions for genotyping assays are available upon request. The genotyping method for the *MC1R* variants was described previously [29].

Statistical methods

We used the χ^2 test to assess whether the genotypes for all fifteen SNPs were in Hardy-Weinberg equilibrium among the controls.

The *MC1R* gene has been strongly associated with human pigmentary phenotypes, especially with red hair color [32–34]. We previously reported the frequency distribution of seven common *MC1R* variants among controls, including three "red hair color" (RHC) variants (Arg151Cys, Arg160Trp, and Asp294His) and four "non-red hair color" (NRHC) variants (Val60Leu, Val92Met, Ile155Thr, and Arg163Gln) [29]. In order to compare the contribution of these fifteen SNPs to pigmentary phenotypes with that of the *MC1R* variants, we evaluated the associations between the *MC1R* variants and pigmentary phenotypes in parallel. We regressed an ordinal coding for skin color (1=fair; 2=medium; and 3=olive) or tanning ability (1=practically none; 2=light tan; 3=average tan; and 4=deep tan) on an ordinal coding for genotype (0, 1, or 2 copies of SNP minor allele). For hair color, we used two different statistical models: A) we tested the association between the ordinal genotype coding and an ordinal coding of hair color excluding the women with red hair (1=black; 2=dark brown; 3=light brown; and 4=blonde) using linear regression; and B) we used logistic regression to test the association between the ordinal genotype coding and a binary red hair phenotype (red hair vs. non-red hair color). For the *SLC45A2* Gln272Lys and three *MC1R* NRHC variants (Val92Met, Ile155Thr, and Arg163Gln), we used Fisher's exact test for "red vs. non-red hair color" analysis because none of the women with red hair color carried the variant allele.

We evaluated the association between each genotype and skin cancer risk using unconditional logistic regression. We compared each type of skin cancer with the common control series to increase the statistical power.

In the haplotype analysis, haplotype frequencies and expected haplotype counts for each individual were estimated using a simple expectation-maximization algorithm, as implemented in SAS PROC HAPLOTYPE. The association analyses between haplotypes and binary pigmentary phenotypes and skin cancer risk were performed using the expectation-substitution technique [35]. All statistical analyses were two-sided and carried out using SAS V9.1 (SAS Institute, Cary, NC).

Results

Descriptive characteristics of cases and controls

At the beginning of the follow-up of this nested case-control study, the women were between 43 and 68 years old with a mean age of 58.7. The mean age at diagnosis of melanoma cases was 63.4 and that of SCC and BCC cases was 64.7 and 64.0, respectively. Basic characteristics of cases and controls in this study are presented in Table 1. Detailed description and statistical tests were published previously [23]. Briefly, skin cancer cases were more likely to possess red hair color and fair skin color. The childhood tanning ability of cases was less than that of controls. Women in the West and South regions were more likely to be diagnosed with SCC or BCC compared with those in Northeast. A family history of skin cancer was a risk factor for the three types of skin cancer. Those with skin cancers were more likely to have used sunlamps or attended tanning salons. Those with skin cancers had higher cumulative sun exposure while wearing a bathing suit and more lifetime severe sunburns that blistered.

Association between the fifteen SNPs in pigmentation genes and pigmentary phenotypes

Information on the fifteen SNPs in pigmentation genes is presented in Table 2. We selected putative functional SNPs in the 6 pigmentation genes (*TYR, OCA2, SLC45A2, POMC, ASIP*, and *ATRN*), including non-synonymous SNPs and those in the promoter and UTR regions. For

the *SLC24A5* gene, the Ala111Thr (rs1426654) is monomorphic in HapMap CEU samples. The SNP rs17426596 is the only one with minor allele frequency >1% in the HapMap CEU samples. Recently, an eye-color variant in *TYRP1* (rs1408799) was reported to be associated with melanoma risk [25, 27]. A haplotype near *ASIP* carrying rs4911414 variant allele[T] and rs1015362 major allele[G] (hereafter called *ASIP* AH) was associated with pigmentary phenotypes (skin sensitivity to sun, burn, and freckle) as well as the risks of melanoma and BCC [25, 27]. These three SNPs were evaluated for the association with pigmentary phenotypes and the risk of skin cancer as well in this study. The distributions of genotypes for these fifteen SNPs were in Hardy-Weinberg equilibrium among controls. The participants of this study were from 11 states, which were grouped into three regions (Northeast, Northcentral, and West and South). The minor allele frequencies of the fifteen genetic variants according to the 11 individual states are presented in Supplementary Table 1. There were no significant differences in minor allele frequencies of these fifteen SNPs across the 11 states (all *p*-values>0.05).

We evaluated the associations between the fifteen SNPs and pigmentary phenotypes including hair color, skin color, and tanning ability among controls (Table 3). We observed significant associations between *SLC45A2* Phe374Leu (*p*-value $=2.4\times10^{-7}$) and *SLC45A2* Glu272Lys (*p*-value $=6.0\times10^{-5}$), and hair color (black to blonde). We then mutually adjusted these two significant *SLC45A2* SNPs and found that only the *SLC45A2* Phe374Leu remained significant. The *p*-value for *SLC45A2* Phe374Leu and *SLC45A2* Glu272Lys was 8.0×10^{-4} and 0.15, respectively. The *SLC45A2* Phe374Leu remained significantly associated with hair color after adjusting for *MC1R* variants (*p*-value $=3.3\times10^{-6}$) (Supplementary Table 2a and 2b). However, in the analysis of "red hair vs. non-red hair color", there was no significant association between any of the fifteen polymorphisms and red hair color (Table 3). In contrast, the three *MC1R* RHC variants were significantly associated with red hair color phenotype.

We found that the *TYR* Arg402Gln and *SLC45A2* Phe374Leu were significantly associated with skin color (*p*-value $=7.7\times10^{-4}$ and 1.1×10^{-7}, respectively) and tanning ability (*p*-value $=7.3\times10^{-4}$ and 2.5×10^{-4}, respectively). The *OCA2* Arg305Trp was associated with skin color (*p*-value=0.04). These associations remained significant after controlling for the *MC1R* variants (Table 3, Supplementary Table 2a and 2b). The significant associations between the *SLC45A2* Glu272Lys and skin color and tanning ability were eliminated after controlling for the *SLC45A2* Phe374Leu (*p*-value =0.97 and 0.49, respectively).

We found a significant association of *ASIP* G>T (rs4911414) polymorphism with skin color and tanning ability (*p*-value $=1.4\times10^{-3}$ and 0.02, respectively). However, the association with tanning ability was eliminated after adjusting for the *MC1R* variants (*p*-value =0.19), while the association with skin color remained significant (*p*-value =0.01) (Table 3, Supplementary Table 2a and 2b). We also performed a global test to evaluate whether the haplotype frequencies were different between various pigmentary phenotypes (Supplementary Table 3). Consistent with the single SNP analysis of *ASIP* G>T (rs4911414), 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). This association remained significant after adjusting for the *ASIP* g.8818 A>G (OR, 2.54; 95% CI, 1.58–4.07).

All the significant associations described above with pigmentary phenotypes remained significant after controlling for the geographic regions (either by 11 states or 3 combined groups) (data not shown).

Association between the fifteen SNPs in pigmentation genes and skin cancer risk

The main effect of each polymorphism was evaluated across the three types of skin cancer (Table 4). In the analyses controlling for age, we observed that the *TYRP1* rs1408799 and *SLC45A2* -1721 C>G were associated with melanoma risk (OR, 0.77; 95% CI, 0.60–0.98 and

OR, 0.75; 95% CI, 0.60–0.95, respectively); the *TYR* Ser192Tyr and *ASIP* rs4911414 were associated with SCC risk (OR, 1.23; 95% CI, 1.00–1.50 and OR, 1.29; 95% CI, 1.05–1.59, respectively); the *OCA2* Arg419Gln and *ASIP* g.8818 A>G were associated with BCC risk (OR, 1.50; 95% CI, 1.06–2.13 and OR, 0.73; 95% CI, 0.53–1.00, respectively). These associations remained similar after adjusting for either pigmentary phenotypes (hair color, skin color, and tanning ability) (Supplementary Table 4) or *MC1R* variants (Supplementary Table 5a and 5b) or skin cancer risk factors including constitutional susceptibility score (tertiles), family history of skin cancer, the number of lifetime severe sunburns that blistered, sunlamp use or tanning salon attendance, cumulative sun exposure while wearing a bathing suit, and geographic regions (either by 11 states or 3 combined groups) (data not shown). None of those significant associations with skin cancer risk remained significant after the Bonferroni correction (all *p*-values >0.05/45 (15 SNPs and 3 types of skin cancer) =0.001).

Haplotypes for the *TYR*, *OCA2*, *SLC45A2*, and *ASIP* genes and skin cancer risk

We performed the global test to evaluate the difference in haplotype frequencies between cases and controls (Table 5). We found significant differences in *TYR* haplotype frequency for SCC (*p*-value =0.007), *OCA2* haplotype frequency for BCC (*p*-value =0.03), and *ASIP* haplotype frequencies for melanoma and SCC (*p*-value =0.008 and 0.004, respectively). For the *TYR* gene, the haplotypes that carried only one variant allele at the two sites were significantly associated with an increased risk of SCC. The adjusted ORs (95% CI) for the haplotype carrying only the Ser192Tyr or only the Arg402Gln variant alleles were 1.48 (1.16–1.89) and 1.35 (1.04–1.74), respectively. The Arg402Gln variant was not significantly associated with risk of SCC in the single SNP analysis. We observed that the haplotype carrying *OCA2* Arg419Gln variant allele and the *OCA2* Arg305Trp major allele was significantly associated with an increased risk of BCC (adjusted OR, 1.62; 95% CI, 1.13–2.32). For the *ASIP* gene, the haplotype AH was significantly associated with an increased risk of melanoma (OR, 1.68; 95% CI, 1.18–2.39) and SCC (OR, 1.54; 95% CI, 1.08–2.19). The *ASIP* rs4911414 variant allele was not significantly associated with melanoma risk in the single SNP analysis.

Power calculation

The Quanto statistical software version 1.2.3 was used for power calculation [36]. We calculated the power to detect the specified ORs at various allele frequencies of variant allele in additive models. The calculations were based on a two-sided alpha of 0.05, and the particular sizes of different phenotypic population groups presented in the Table 1 of this study. For melanoma (SCC or BCC), we have 80% power to detect an OR of 1.80 (1.72 or 1.70), 1.48 (1.42 or 1.41), and 1.35 (1.32 or 1.31) if the minor allele frequency is 5%, 15%, and 40%, respectively. For pigmentary phenotypes, we calculated the power to detect the difference between dark and light pigmentation: black/brown and blonde hair color, medium/olive and fair skin color, and average/deep tan and practically none/light tan. For hair color (skin color or tanning ability), we have 80% power to detect an OR of 2.26 (1.99 or 1.90), 1.71 (1.52 or 1.50), and 1.52 (1.36 or 1.35) if the minor allele frequency is 5%, 15%, and 40%, respectively.

Discussion

Hair color and skin color show striking variations between human subgroups. The dark pigmentation and tanning response protect the skin from UV [37]. To date, although more than 100 candidate pigmentation genes containing common genetic variants have been identified, only the variants in the *MC1R* gene have been consistently implicated in the variation of pigmentary phenotypes as well as skin cancer risk [5, 29, 34, 38–40]. In our study only the three *MC1R* RHC variants were significantly associated with red hair color, supporting the major contribution of the *MC1R* gene to the red hair color phenotype, an autosomal recessive trait [28, 29, 33, 41]. In addition, some genetic variants in the other pigmentation genes showed

significant associations with non-red hair color (black to blonde) in this study, suggesting distinct mechanisms in the formation of non-red hair color and red hair color. Previous studies reported possible associations between some genetic variants evaluated in this study and pigmentary phenotypes. We summarized these studies in Supplementary Table 6.

TYR Arg402Gln, a common polymorphism of tyrosinase, was correlated with reduced pigmentation of the retina and iris resulting from low tyrosinase activity [42]. In addition to the associations of this SNP with skin color and tanning ability observed in this study, Sulem et al. reported that this SNP was associated with eye color and possibly with blond hair color [28]. Mutations in murine *SLC45A2* gene lead to hypopigmentation of the eyes and fur [43]. Two non-synonymous SNPs in this gene, *SLC45A2* Phe374Leu and *SLC45A2* Glu272Lys, were associated with darker pigmentary phenotypes in our study, which is consistent with a previous report [44]. However, our multivariate analysis mutually adjusting for these two SNPs showed that the effect of *SLC45A2* Glu272Lys on pigmentary phenotypes was explained by the variant *SLC45A2* Phe374Leu. For the promoter polymorphism *SLC45A2* -1721 C>G, we did not detect any significant associations with pigmentary traits, while a previous study reported an association between this SNP and olive skin color [45].

The deletion of *OCA2* gene has been linked to reduced pigmentation of skin, hair, and eyes in Prader-Willi Syndrome [46]. Two genetic variants in this gene, Arg305Trp and Arg419Gln, have been correlated with dark eye color [47]. However, these two SNPs were not associated with pigmentary phenotypes measured in our study, such as hair color, skin color, and tanning ability except that the Arg305Trp was marginally associated with skin color. A polymorphism in the 3' untranslated region of *ASIP* gene (g.8818 A>G) has been previously reported to be associated with dark pigmentary phenotypes among populations of African Americans or European-ancestry [48–51]. Although we did not find a significant association of this SNP with any of the pigmentary phenotypes, our haplotype analysis showed that the haplotype *ASIP* AH was significantly associated with fair skin color and this association remained significant after adjusting for *ASIP* g.8818 A>G. Similarly, Sulem et al. reported that the *ASIP* AH haplotype remained significant for the pigmentary traits, such as burning and freckling, after adjusting for *ASIP* g.8818 A>G, while the association of *ASIP* g.8818 A>G with pigmentary traits were eliminated after adjusting for the haplotype [27].

We evaluated the contributions of genetic variants in the pigmentation genes not only to pigmentary phenotypes but also to the risks of three types of skin cancers among U.S. Caucasians, whereas most previous studies only evaluated the relation of those genetic variants to the pigmentary traits. Ours is the first report evaluating the association between genetic variants in the pigmentation genes and the three types of skin cancer simultaneously. We summarized the results from previous studies assessing the associations of the SNPs evaluated in this study with melanoma risk in Supplementary Table 7. Only one previous study examined pigmentation genes with BCC risk [25]. Overall, in this study, the associations observed with an altered risk of at least one skin cancer in the single SNP analysis remained similar after adjusting for either pigmentary phenotypes or *MC1R* variants, suggesting that these genetic variants play a role in development of skin cancer beyond their influence on pigmentary phenotypes. Furthermore, most of these genetic variants were not associated with the pigmentary phenotypes. The genes involved in pigmentation process may also contribute to other cellular responses to UV exposure. For example, the immune and inflammatory responses to UV exposure are at least partially mediated by the *MC1R* gene [52–54]. Also, the *OCA2* gene increases cellular sensitivity to toxic compounds in addition to its role in controlling melanosome biogenesis [55]. In addition, tyrosinase is recognized as melanoma-associated antigen by cytotoxic T lymphocytes [56]. It is therefore plausible that these genetic variants associated with skin cancer risks may influence other cellular responses leading to skin cancer development. However, we cannot rule out the possibility that the associations with skin cancer

risk could be due to chance considering the number of tests performed. Therefore, we should be cautious when interpreting the results on skin cancer risks.

Recently, Gudbjartsson et al. reported a significant association of the *TYR* 402Gln variant and the *ASIP* AH haplotype with the increased risks of melanoma and BCC [25]. The haplotype analysis performed in our study showed that the risk estimate associated with the *TYR* 402Gln was elevated for melanoma (OR, 1.29; 95% CI, 0.97–1.71) and SCC (OR, 1.35; 95% CI, 1.04–1.74), which was not observed in the single SNP analysis. For the *ASIP* AH haplotype, in addition to the association with an increased risk of melanoma, we also found significant association with an increased risk of SCC. The inverse association between the *OCA2* Arg305Trp polymorphism and melanoma risk among French Caucasians reported by Jannot et al. is inconsistent with the result of our study [57]. We did not find any significant associations between this SNP and three types of skin cancer risks in this study.

Two of the four regions that we found to be associated with variation in pigmentary phenotypes among Europeans (*SLC45A2* and *OCA2*) show strong evidence of recent positive selection, based on a comparison of allele frequencies across samples from three continental populations (Africa, Asia, and Europe) [58]. Allele frequencies for the *SLC45A2* Phe374Leu polymorphism have been shown to vary greatly across continental populations [58], and less drastically within Europe [59]. The *TYR* SNP rs1126809 Arg402Gln also shows significant differences in allele frequency across the HapMap CEU, CHB, JPT, and YRI panels. This SNP and its surrogate SNP rs1393350 were monomorphic in CHB, JPT, and YRI panels (the minor allele was absent from these samples).

Eight SNPs out of the 15 SNPs were either genotyped as part of the Cancer Genetic Markers of Susceptibility (CGEMS) breast cancer genome-wide association scan or could be imputed with high confidence using the observed genotypes and the HapMap phased data [60]. The CGEMS breast cancer scan consists of 1200 cases of breast cancer and 1200 controls from the Nurses' Health Study of European ancestry genotyped using the Illumina 500k HumanHap platform [61]. In order to assess the potential for within-Europe population stratification bias, we examined the association between these 8 SNPs and the top principal components of genetic variation inferred from the CGEMS genome-wide scan data [62, 63]. For example, the *TYR* rs1393350 SNP, which was strongly associated with skin color and tanning ability in the skin cancer controls, was also significantly associated with two of the top 10 principal components of genetic variation (Supplementary Table 8), suggesting that allele frequency for this marker also vary among European populations. This SNP was strongly associated with tanning ability (information on the skin color is not recorded in the CGEMS data) in the CGEMS genome-wide association study samples (p-value $=5.8\times10^{-11}$), and this association remained significant after adjusting for the top four principal components (p-value $=8.0\times10^{-8}$). In fact, only 3 of the top ten principal components were associated with tanning ability, and together these explained 4.5% of the residual variation in tanning ability, while the *TYR* rs1126809 (a surrogate for Arg402Gln) polymorphism explained 1.5% of the residual variation in tanning ability beyond the effect of top 3 principal components. We believe it is unlikely that the strong associations we see between these markers and pigmentary phenotypes are solely due to population stratification bias. Rather it is likely that differences in the distribution of pigmentary phenotypes across Europe are due in part to differences in allele frequencies at these loci and other as-yet-unknown loci.

Population stratification may be a particularly important issue in assessment of the association of the pigmentation genetic polymorphism with skin cancer risk because of the variation of the allele frequency and the predominance of the disease among light pigmented people, even among European populations. However, the ancestry informative variants mentioned above were not associated with any type of skin cancer, suggesting that the associations of other

variants with skin cancer risk is not due to bias rooted in stratification, a possibility that is also made less likely by the fact that these associations remained significant after controlling for the geographic regions (either by 11 states or 3 combined groups). However, these modest associations require further replication in other populations.

One limitation of this study would be the modest statistical power. It is possible that the modest effects of some genetic variants cannot be detected due to insufficient statistical power. Another limitation of our study was that we used self-reported pigmentary phenotypes. Such assessment may miss certain aspects of pigmentary phenotypes influenced by these genetic variants, such as melanin content and composition [64, 65]. In addition, misclassification is always a concern in epidemiologic studies. The high education level and interest in health of cohort members allows high quality information to be collected. Test-retest reliability of collecting phenotypic factors from questionnaires is moderate to substantial, including skin color, tanning/burning tendency, and sunburn history [66–68].

In conclusion, our study evaluated the associations between genetic variants in the pigmentation genes and pigmentary phenotypes and skin cancer risk. As this reported is one of the very few studies examining such associations, additional studies are warranted to confirm these associations. This information may be useful in understanding the involvement of different pigmentation genes in Caucasian pigmentary phenotypes and skin cancer risk.

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Abbreviations

BCC
: basal cell carcinoma

SCC
: squamous cell carcinoma

CI
: confidence interval

OR
: odds ratio

UV
: ultraviolet

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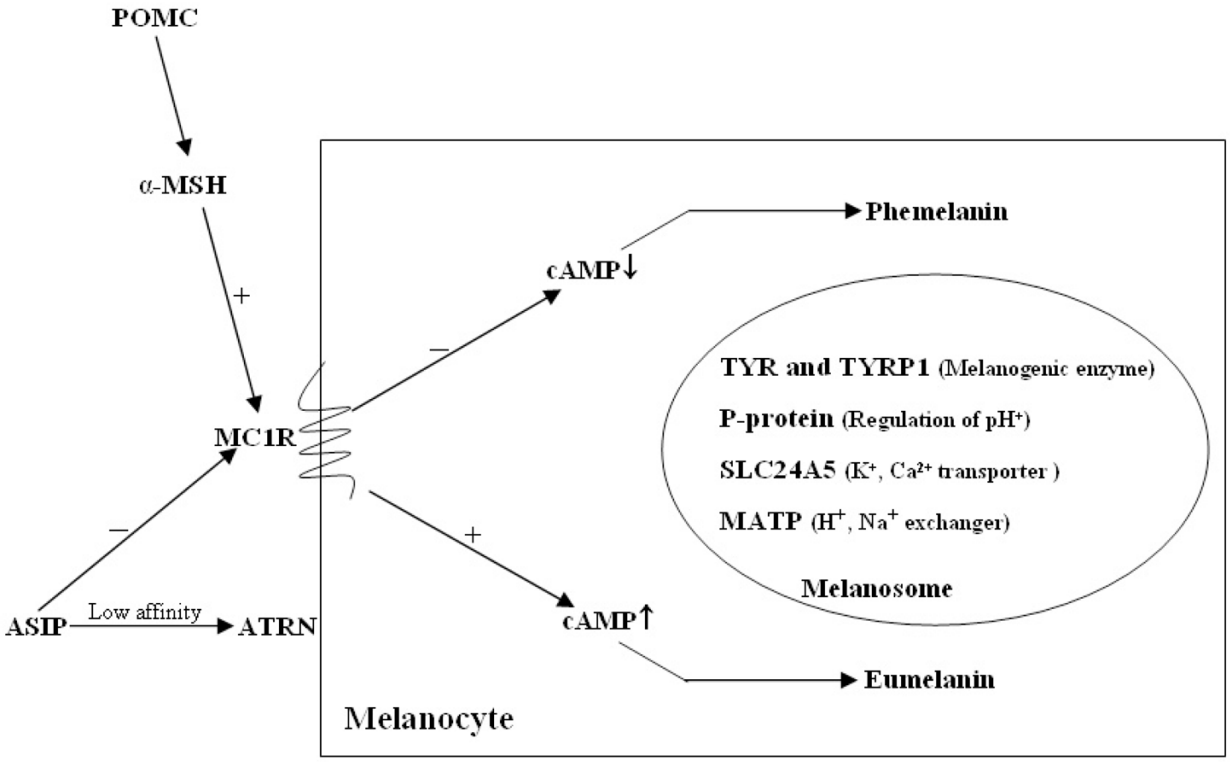

Figure 1. The function of select pigmentation genes in the pigmentation pathway

Induction of POMC/α-MSH activates the *MC1R*, inducing cAMP production in melanocyte. This elevated signaling leads to eumelanin production, resulting in the maturation of the pheomelanosome to the eumelanosome. Attractin (*ATRN*) is a low-affinity receptor for agouti signaling protein (*ASIP*), an antagonist of *MC1R*. Some proteins, such as TYR, TYRP1, P-protein, SLC24A5, and MATP, are involved in inducing melanization of pheomelanosome or eumelanosome, as described in the text.

Table 1

Characteristics of skin cancer cases and controls in the nested case-control study

Characteristic	Controls (n=870)	Melanoma cases (n=218)	SCC cases (n=285)	BCC cases (n=300)
Age at diagnosis (mean, years)	64.5	63.4	64.7	64.0
Natural hair color at age 20 (%)				
Black or dark brown	43.9	31.5	41.3	30.3
Light brown	40.0	42.5	34.6	45.7
Blonde	12.0	15.5	16.8	18.0
Red	2.9	10.5	5.2	4.7
Natural skin color (%)				
Fair	40.0	57.1	54.6	53.0
Medium	36.7	25.6	32.2	31.3
Olive	4.8	0.9	1.8	1.3
Tanning ability (%)				
Practically none	8.3	13.8	13.9	11.7
Light tan	21.1	29.5	24.1	25.5
Average tan	47.3	40.0	47.4	46.5
Tan	23.3	16.7	14.7	16.3
Geographic region at baseline (%)				
Northeast	55.2	58.0	51.7	49.3
Northcentral	23.4	16.9	17.1	20.3
West and South	21.4	25.1	31.1	30.3
Family history of skin cancer (%)	25.1	36.5	35.7	42.7
Sunlamp use or tanning salon attendance (%)	10.0	19.2	14.3	14.7
Highest tertile of cumulative sun exposure with a bathing suit (%)	33.4	53.3	46.1	42.6
Number of lifetime severe sunburns (mean)	5.4	9.6	7.8	8.2

The percentages may not sum to 100 due to rounding.

Table 2

Fifteen SNPs in the selected pigmentation genes

SNP	rs#	Gene	Protein	MAF - controls (%) [b]	MAF - CEU (%) [c]	MAF - CHB/JPT (%) [d]	MAF - YRI (%) [e]
TYR Ser192Tyr	rs1042602	TYR	Tyrosinase	35	42	0	0
TYR Arg402Gln	rs1126809[a]	TYR	Tyrosinase	-	22	0	0
TYR -6895 G>A	rs1393350[a]	TYR	Tyrosinase	28	19	0	0
TYRP1 C>T	rs1408799	TYRP1	Tyrosinase-related protein 1	32	30	98	78
OCA2 Arg305Trp	rs1800401	OCA2	P-protein	6	-	-	-
OCA2 Arg419Gln	rs1800407	OCA2	P-protein	6	7	0	0
SLC45A2 -1721 C>G	rs13289	SLC45A2	MATP	40	32	34	73
SLC45A2 Glu272Lys	rs26722	SLC45A2	MATP	2	0	40	5
SLC45A2 Phe374Leu	rs16891982	SLC45A2	MATP	4	2	99	100
SLC24A5 intron2 T>C	rs17426596	SLC24A5	SLC24A5	4	3	0	0
*POMC*3′ UTR C>T	rs1042571	POMC	POMC, MSH, ACTH	19	-	-	-
*ASIP*G>T	rs4911414	ASIP	Agouti signaling protein	31	28	19	13
*ASIP*G>A	rs1015362	ASIP	Agouti signaling protein	27	23	19	83
*ASIP*g.8818 A>G	rs6058017	ASIP	Agouti signaling protein	13	-	-	-
ATRN Ile426Thr	rs17782078	ATRN	Attractin	5	4	0	0
ATRN Arg1152Lys	rs3886999	ATRN	Attractin	6	4	0	0

[a] The SNP rs1126809 failed the assay and the rs1393350 was genotyped instead (D′=1 and r2=0.86).

[b] Minor allele frequency (MAF) was calculated among controls in this study.

[c] MAF was based on the HapMap CEU (Utah residents with ancestry from northern and western Europe) samples.

[d] MAF was based on the HapMap CHB and JPT (CHB: Han Chinese in Beijing, China; JPT: Japanese in Tokyo, Japan) samples.

[e] MAF was based on the HapMap YRI (Yoruba in Ibadan, Nigeria) samples.

Table 3

Associations between the fifteen SNPs in the selected pigmentation genes and pigmentary phenotypes among controls

SNP	Hair color (black to blonde)			Hair color (red vs. nonred)			Skin color			Tanning ability		
	β	SE	*p*-value	β	SE	*p*-value	β	SE	*p*-value	β	SE	*p*-value
TYR Ser192Tyr	0.08	0.04	0.06	−0.26	0.33	0.42	0.06	0.04	0.10	0.00	0.05	0.97
TYR Arg402Gln**	0.00	0.04	0.92	−0.31	0.35	0.38	−0.12	0.04	7.7E-04	−0.16	0.05	7.3E-04
TYRP1 C>T (rs1408799)	0.02	0.04	0.60	0.32	0.30	0.28	0.02	0.04	0.65	0.04	0.05	0.46
OCA2 Arg305Trp	−0.08	0.08	0.31	0.11	0.62	0.85	0.15	0.07	0.04	0.02	0.10	0.84
OCA2 Arg419Gln	−0.09	0.08	0.23	−1.05	1.01	0.30	−0.10	0.07	0.18	−0.05	0.09	0.61
SLC45A2 -1721 C>G	0.03	0.04	0.49	−0.11	0.30	0.71	0.02	0.03	0.64	0.04	0.04	0.33
SLC45A2 Glu272Lys	−0.58	0.14	6.0E-05	-	-	1.00*	0.44	0.13	1.1E-03	0.48	0.18	7.0E-03
SLC45A2 Phe374Leu	−0.49	0.10	2.4E-07	0.51	0.60	0.39	0.48	0.09	1.1E-07	0.42	0.11	2.5E-04
SLC24A5 intron2 T>C	0.11	0.10	0.26	0.97	0.54	0.08	−0.08	0.08	0.34	−0.03	0.12	0.78
POMC 3′ UTR C>T	−0.06	0.05	0.23	0.09	0.36	0.79	−0.03	0.04	0.52	−0.01	0.06	0.86
ASIP G>T (rs4911414)	0.02	0.04	0.68	0.33	0.30	0.27	−0.11	0.03	1.4E-03	−0.11	0.05	0.02
ASIP G>A (rs1015362)	−0.06	0.04	0.15	−0.37	0.35	0.29	−0.01	0.04	0.76	0.00	0.05	0.96
ASIP g.8818 A>G	−0.01	0.06	0.81	0.07	0.44	0.88	0.00	0.05	0.98	0.08	0.07	0.25
ATRN Ile426Thr	0.04	0.08	0.65	0.50	0.54	0.35	−0.07	0.07	0.31	−0.02	0.10	0.87
ATRN Arg1152Lys	0.05	0.08	0.50	0.45	0.53	0.40	−0.08	0.07	0.24	−0.02	0.10	0.82
***MC1R* variants**												
MC1R Val60Leu	−0.03	0.05	0.63	−1.91	1.01	0.06	−0.06	0.05	0.23	−0.13	0.06	0.05
MC1R Val92Met	−0.13	0.06	0.04	-	-	0.01*	0.00	0.06	1.00	−0.10	0.07	0.18
MC1R Arg151Cys	0.17	0.08	0.03	2.21	0.40	2.6E-08	−0.24	0.06	1.4E-04	−0.46	0.09	1.4E-07
MC1R Ile155Thr	0.09	0.16	0.58	-	-	1.00*	−0.05	0.13	0.71	−0.18	0.19	0.34
MC1R Arg160Trp	0.20	0.07	5.9E-03	2.14	0.34	5.0E-10	−0.19	0.06	9.4E-04	−0.28	0.08	4.1E-04
MC1R Arg163Gln	0.05	0.09	0.58	-	-	0.25*	0.01	0.09	0.92	−0.07	0.11	0.54
MC1R Asp294His	0.27	0.16	0.08	1.94	0.59	9.6E-04	−0.37	0.13	4.2E-03	−0.67	0.18	2.1E-04

The regression parameter beta refers to the mean change in scoring in hair color (black to blonde), skin color, and tanning ability (or change in log odds of red hair for red hair analyses) per copy of the SNP minor allele.

*Fisher's exact test was used. The β value was not calculated because none of the women with red hair color carried the variant allele.

** TYR -6895 G>A (rs1393350) was genotyped instead.

Table 4

Associations between the fifteen SNPs in the selected pigmentation genes and skin cancer risk

SNP	Melanoma		SCC		BCC	
	Additive OR*	p for trend	Additive OR*	p for trend	Additive OR*	p trend
***TYR* Ser192Tyr**	1.18 (0.94–1.48)	0.15	1.23 (1.00–1.50)	0.05	1.08 (0.89–1.33)	0.43
TYR* Arg402Gln*	1.05 (0.83–1.32)	0.71	1.07 (0.87–1.32)	0.52	1.04 (0.84–1.27)	0.74
***TYRP1* C>T (rs1408799)**	0.77 (0.60–0.98)	0.03	0.96 (0.78–1.18)	0.71	0.95 (0.77–1.16)	0.60
***OCA2* Arg305Trp**	0.92 (0.56–1.52)	0.76	0.87 (0.56–1.36)	0.55	0.97 (0.63–1.50)	0.91
***OCA2* Arg419Gln**	1.33 (0.89–2.01)	0.17	1.39 (0.97–2.01)	0.07	1.50 (1.06–2.13)	0.02
***SLC45A2*-1721 C>G**	0.75 (0.60–0.95)	0.01	1.08 (0.89–1.31)	0.42	0.91 (0.75–1.11)	0.36
***SLC45A2* Glu272Lys**	1.19 (0.53–2.67)	0.68	0.55 (0.21–1.45)	0.23	1.04 (0.49–2.17)	0.93
***SLC45A2* Phe374Leu**	0.66 (0.34–1.29)	0.22	0.76 (0.43–1.34)	0.34	0.61 (0.33–1.11)	0.10
***SLC24A5* intron2 T>C**	0.75 (0.39–1.43)	0.38	0.86 (0.50–1.48)	0.58	0.80 (0.47–1.38)	0.43
***POMC* 3′ UTR C>T**	0.99 (0.74–1.31)	0.92	0.95 (0.74–1.22)	0.68	0.95 (0.75–1.22)	0.71
***ASIP* G>T (rs4911414)**	1.21 (0.96–1.51)	0.10	1.29 (1.05–1.59)	0.01	1.16 (0.95–1.42)	0.14
***ASIP* G>A (rs1015362)**	0.89 (0.69–1.13)	0.34	1.14 (0.92–1.41)	0.23	1.06 (0.86–1.31)	0.59
***ASIP* g.8818 A>G**	0.89 (0.64–1.24)	0.50	0.79 (0.58–1.09)	0.15	0.73 (0.53–1.00)	0.05
***ATRN* Ile426Thr**	0.87 (0.52–1.45)	0.59	1.14 (0.75–1.72)	0.54	1.08 (0.72–1.62)	0.72
***ATRN* Arg1152Lys**	0.86 (0.53–1.42)	0.56	1.09 (0.72–1.63)	0.69	1.09 (0.73–1.61)	0.67

*Additive OR was calculated based on the unconditional logistic regression adjusted for the age.

**TYR -6895 G>A (rs1393350) was genotyped instead.

Table 5

Haplotypes for SNPs in the *TYR*, *OCA2*, *SLC45A2*, and *ASIP* genes and skin cancer risk

TYR				**Melanoma**		**SCC**		**BCC**	
		Controls		**Cases**		**Cases**		**Cases**	
A	**B**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	0	618	38.0	136	32.5	164	31.2	206	37.0
		Multivariate OR		1.00		1.00		1.00	
1	0	551	33.9	157	37.6	206	39.4	190	34.2
		Multivariate OR		1.34 (1.03–1.75)		1.48 (1.16–1.89)		1.05 (0.83–1.34)	
0	1	438	26.9	122	29.2	150	28.7	153	27.6
		Multivariate OR		1.29 (0.97–1.71)		1.35 (1.04–1.74)		1.06 (0.83–1.35)	
1	1	19	1.2	3	0.7	4	0.7	7	1.2
		Multivariate OR		0.43 (0.07–2.79)		0.34 (0.05–2.30)		0.89 (0.32–2.51)	

A: Ser192Tyr; B: Arg402Gln[a]

OCA2				**Melanoma**		**SCC**		**BCC**[c]	
		Controls		**Cases**		**Cases**		**Cases**	
A	**B**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	0	1417	88.3	351	87.3	468	86.7	465	85.2
		Multivariate OR		1.00		1.00		1.00	
0	1	94	5.9	31	7.7	44	8.1	51	9.3
		Multivariate OR		1.33 (0.88–2.03)		1.40 (0.97–2.03)		1.62 (1.13–2.32)	
1	0	93	5.8	20	5.0	28	5.2	30	5.5
		Multivariate OR		0.91 (0.54–1.51)		0.91 (0.58–1.41)		0.99 (0.64–1.53)	

A: Arg305Trp; B: Arg419Gln

SLC45A2			Controls		Melanoma Cases		SCC Cases		BCC Cases	
A	**B**	**C**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	0	0	966	59.4	268	65.5	312	57.4	357	62.1
			Multivariate OR		1.00		1.00		1.00	
1	0	0	601	37.0	130	31.6	216	39.7	203	35.4
			Multivariate OR		0.77 (0.61–0.97)		1.11 (0.91–1.35)		0.93 (0.76–1.13)	
1	0	1	23	1.4	4	0.9	8	1.5	4	0.7
			Multivariate OR		0.54 (0.16–1.84)		1.17 (0.48–2.84)		0.48 (0.16–1.47)	
Rare < 1%			36	2.2	8	2.0	8	1.5	10	1.7
			Multivariate OR		0.84 (0.38–1.86)		0.66 (0.29–1.50)		0.75 (0.36–1.57)	

A: -1721C>G; B: Glu272Lys; C: Phe374Leu

ASIP			Controls		Melanoma[d] Cases		SCC[e] Cases		BCC Cases	
	A	**B**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
	0	0	1020	65.7	253	63	294	60.8	337	63.1
			Multivariate OR		1.00		1.00		1.00	
	1	1	366	23.6	91	22.6	132	27.3	134	25.1
			Multivariate OR		0.99 (0.76–1.29)		1.25 (0.99–1.58)		1.09 (0.87–1.38)	
AH[f]	1	0	120	7.7	52	13.0	52	10.7	48	9.0
			Multivariate OR		1.68 (1.18–2.39)		1.54 (1.08–2.19)		1.22 (0.86–1.74)	
	0	0	48	3.1	6	1.6	6	1.2	15	2.8
			Multivariate OR		0.52 (0.21–1.29)		0.43 (0.17–1.08)		1.00 (0.54–1.83)	

A: rs4911414; B: rs1015362

0, common allele; 1, rare allele; Logistic regression adjusted for the age.

[a]TYR -6895 G>A (rs1393350) was genotyped instead;

[b] p-value for global test for SCC is 0.007;

[c] p-value for global test for BCC is 0.03;

[d] p-value for global test for melanoma is 0.008;

[e] p-value for global test for SCC is 0.004;

[f] AH means the ASIP haplotype carrying the rs4911414 variant allele [T] and the rs1015362 major allele [G].