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Genome-wide association study of tanning phenotype in a population of European ancestry

Hongmei Nan[1,2,3], **Peter Kraft**[2,3], **Abrar A. Qureshi**[1], **Qun Guo**[1,3], **Constance Chen**[2,3], **Susan E. Hankinson**[1,2], **Frank B. Hu**[1,2,4], **Gilles Thomas**[5], **Robert N. Hoover**[5], **Stephen Chanock**[5], **David J. Hunter**[1,2,3,4,5,6], and **Jiali Han**[1,3]

[1] Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA

[2] Department of Epidemiology, Harvard School of Public Health, Boston, MA

[3] Program in Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston, MA

[4] Department of Nutrition, Harvard School of Public Health, Boston, MA

[5] Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD

[6] Broad Institute of Harvard and MIT, Cambridge, MA

Abstract

We conducted a multi-stage genome-wide association study (GWAS) of tanning response after exposure to sunlight in over 9,000 men and women of European ancestry who live in the United States. An initial analysis of 528,173 single nucleotide polymorphisms (SNPs) genotyped on 2,287 women identified LOC401937 (rs966321) on chromosome 1 as a novel locus highly associated with tanning ability, and we confirmed this association in 870 women controls from a skin-cancer case-control study with joint p-value=1.6×10^{-9}. We further genotyped this SNP in two subsequent replication studies (one with 3,750 women and the other with 2,405 men). This association was not replicated in either of these two studies. We found that several SNPs reaching the genome-wide significance level are located in or adjacent to the loci previously known as pigmentation genes: *MATP*, *IRF4*, *TYR*, *OCA2*, and *MC1R*. Overall, these tanning ability-related loci are similar to those hair color-related loci reported previously in the GWAS of hair color.

Introduction

Human pigmentation shows substantial variation both within and among human populations, with high heritability (Frisancho *et al.*, 1981; Harrison and Owen, 1964). The main contributor to human pigmentation is the melanin synthesized within melanosomes in melanocytes. There are two main types of melanin: pheomelanin (red or yellow) and eumelanin (black or brown). The tanning phenotype, combined with the hair color, skin color, and eye color, represents the visible phenotype of the human pigmentary trait. Tanning is the physiologically stimulated response to ultraviolet (UV) radiation of the solar light. UV exposure increases the production of eumelanin in an attempt to protect the skin from further damage. The sensitivity to UV

*Correspondence to: Hongmei Nan, Program in Molecular and Genetic Epidemiology, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Ave, Boston, MA 02115, USA. Telephone: +617-432-5896. Fax: 617-432-1722. E-mail: hnan@hsph.harvard.edu.

Conflict of Interest
The authors declare no conflict of interest.

radiation on the skin varies substantially both between persons at the same body site and between different body sites on the same person (Ha *et al.*, 2003; Waterston *et al.*, 2004). UV light is the major environmental risk factor for skin cancer in humans. Less tanning response after exposure to UV, along with lighter skin color and hair color, is a host susceptibility risk factor for skin cancer (Han *et al.*, 2006).

It has been hypothesized that human pigmentation is tightly regulated by multiple pigmentation genes harboring a handful of genetic variants. Although more than 100 genes involved in the process of pigmentation, such as maturation, transport, and distribution of melanosomes, have been identified through animal models (Jackson, 1994), only several genes were identified to contain common genetic variants associated with human pigmentation in the normal range (Han *et al.*, 2008; Rees, 2004; Sulem *et al.*, 2008; Sulem *et al.*, 2007). With new technologies that enable analysis of hundreds of thousands of single nucleotide polymorphisms (SNPs), combined with new insights into the structure of variation in the human genome (Frazer *et al.*, 2007), it is now possible to scan the genome in an agnostic manner in search of common genetic variants associated with human pigmentation. We have previously performed a GWAS of natural hair color to identify common genetic variants associated with variation in natural diversity of human pigmentation (Han *et al.*, 2008). In this study, we further conducted a GWAS of tanning response after exposure to UV in 2,287 U.S. women of European ancestry using data on 528,173 SNPs genotyped as part of the Cancer Genetic Markers of Susceptibility breast cancer GWAS (Hunter *et al.*, 2007). Promising SNPs were examined in three additional studies with data on tanning response after exposure to UV: 870 U.S. women controls who were free of diagnosed skin cancer from a skin cancer case-control study within the Nurse's Health Study (NHS); 3,750 U.S. women from a diabetes case-control study within the NHS; and 2,405 U.S. men from a diabetes case-control study within the Health Professional Follow-up Study (HPFS).

Results and Discussion

We compared the distribution of observed p-values from each of the 528,173 SNPs in the GWAS with those expected under the global null that none of the tested SNPs is associated with tanning ability to sunlight (Figure 1). The distribution of the observed p-values for the crude analyses that restricted analysis to women of self-reported European ancestry but did not further adjust for potential population stratification shows evidence for systematic bias: the genomic control inflation factor for the crude analyses (the ratio of the median observed test statistic to the theoretical median) is λ_{GC}=1.14. This systematic bias is most likely due to confounding by latent population stratification. As a major determinant of tanning ability to sunlight, skin color varies along a light-dark gradient from northern to southern Europe, so it will be associated with any SNP marker whose minor allele frequency also varies along a north-south gradient, even if that marker is not in linkage disequilibrium (LD) with a causal tanning locus (Campbell *et al.*, 2005). Adjusting for the top four principal components of genetic variance (Price *et al.*, 2006) eliminated most of the apparent residual confounding due to population stratification (λ_{GC}=1.02 for the adjusted analyses); further control for up to 50 principal components did not alter the λ_{GC}. All of the association results from the initial GWAS reported below are from analyses that adjusted for the top four principal components of genetic variation.

The GWAS identified several genomic locations as potentially associated with tanning response after exposure to UV (Figure 2). Of 528,173 SNPs tested, the 60 SNPs with the most extreme p-values associated with tanning response after exposure to UV are listed in Table 1.

Among the 60 SNPs, we selected 40 SNPs for further study in an independent sample (Table S1). The remaining 20 SNPs were in strong LD (r^2>0.8) with one of these 40 SNPs (Table 1).

Of the 40 SNPs selected, 13 SNPs located in the *MATP*, *IRF4*, *HERC2*/*OCA2*, and *MC1R* genes were previously evaluated in the GWAS of natural hair color by our group (Table 1 and Table S1) (Han *et al.*, 2008). Notably, the most significant loci (rs12203592 in the *IRF4* gene and rs12913832 in the *HERC2*/*OCA2* gene) for hair color were the same as those for tanning ability. It has been shown that the HERC2/OCA2 locus is associated with human pigmentary variation and the SNP rs12913832 in this region has been identified as a determinant for human blue-brown eye color and hair color (Eiberg *et al.*, 2008;Han *et al.*, 2008;Shekar *et al.*, 2008;Sturm *et al.*, 2008). We finally selected 27 SNPs for further study in an independent sample (Table 2). The sample consisted of 870 controls of European ancestry from a nested case-control study of skin cancer within the NHS.

Eight of these 27 SNPs showed evidence of significant associations with tanning ability among the 870 controls ($p<0.05$) (Table 2). Those SNPs were located on chromosome 1 (rs966321), chromosome 5 (rs40132), chromosome 6 (rs12210050), chromosome 11 (rs1393350), chromosome 14 (rs17094273), and chromosome 16 (rs352935, rs464349, and rs11648785), respectively. These eight SNPs showed very strong evidence of associations with tanning ability in a pooled analysis of the initial GWAS and the replication sample ($p<9.5\times10^{-8}=0.05/528{,}173$) (Table 2). Of these eight SNPs, six of them (rs40132, rs12210050, rs17094273, rs352935, rs464349, and rs11648785) were located in or adjacent to the four hair color-related loci reported previously in the GWAS of hair color conducted by our group (Han *et al.*, 2008): *MATP*, *EXOC2*, *SLC24A4*, and *MC1R*. One SNP (rs1393350) in the *TYR* gene was previously found to be associated with skin color and tanning ability from a candidate gene approach by our group. The remaining one novel SNP rs966321 located on chromosome 1 (LOC401937) was strongly associated with tanning ability in the initial GWAS and the follow-up study (pooled p-value for trend = 1.6×10^{-9}). We genotyped rs966321 in an additional 6,155 subjects of predominantly European ancestry from the United States, including 3,750 women from the NHS and 2,405 men from the HPFS. This significant association was not reproduced in subsequent replication studies. The p-values were 0.59 (regression parameter beta (β), −0.01) and 0.16 (β, 0.05), respectively. Using the same three subsequent replication studies, we successfully replicated the associations between hair color and tanning ability and some SNPs identified from our previous GWAS for hair color (Han *et al.*, 2008). It appears that the SNP rs966321 is not a robust variant influencing tanning ability, which further underlines that the replication in independent studies is the key to confirm robust associations in genetic association studies.

We identified the SNP rs40132 in the *MATP* gene from the GWAS, and the association with tanning ability was confirmed in the follow-up study (pooled p-value=1.5×10^{-8}). Three SNPs in the *MATP* gene have been associated with human pigmentation: rs16891982 (Phe374Leu), rs26722 (Glu272Lys), and rs13289 C/G (−1721 in the promoter region) (Graf *et al.*, 2005; Graf *et al.*, 2007). We previously evaluated these three SNPs for associations with pigmentary phenotypes in the controls of skin cancer study. None of the three SNPs were in strong LD with rs40132 ($r^2<0.08$), which is an intronic SNP. A multivariate analysis mutually adjusting for rs40132, rs16891982, rs26722, and rs13289 simultaneously showed that only rs16891982 remained significant in the model (p-value=0.02) and other SNPs became non-significant (p-value>0.05). The MATP, a membrane-associated transporter protein, has been considered as a sodium-hydrogen exchanger of melanosomes, regulating tyrosinase activity in human melanocyte (Smith *et al.*, 2004). These data suggested that rs16891982 is most likely to be the causal variant or in strong LD with the causal variant in the *MATP* gene.

The SNP rs12210050 in the *EXOC2 (SEC5L1)* gene was strongly associated with tanning ability in the initial GWAS and was confirmed in the follow-up study (pooled p-value $=5.5\times10^{-14}$). On the same chromosome 6, 79.2 kb telomeric from the *EXOC2* rs12210050, a SNP (rs12203592) in the intron 4 of the *IRF4* gene, has been strongly associated with

pigmentary phenotypes, such as hair color, tanning ability, and skin color in the GWAS of hair color (Han *et al.*, 2008). These two SNPs are in weak LD (r^2=0.26). We mutually adjusted these two significant SNPs and found that only rs12203592 remained significant (p-value=8.3×10^{-8} in the follow-up study). Sulem et al. identified two SNPs (rs4959270 and rs1540771) between the *EXOC2* and *IRF4* genes in relation to hair color and skin sensitivity to sun with much weaker associations than those of the SNP rs12203592 in the *IRF4* gene (Sulem *et al.*, 2007).

A SNP (rs1393350) in the *TYR* gene showed a significant association with tanning ability in the initial GWAS and was confirmed in the follow-up study (pooled p-value=2.4×10^{-13}). On the same chromosome 11, we identified two SNPs (*TYR* rs10830236 and *GRM5* rs10831496) associated with tanning ability in the initial GWAS, but not in the follow-up study. Neither of these two SNPs, rs10831496 and rs10830236, was in the LD with rs1393350 (r^2=0.06 and 0.64, respectively). Only rs1393350 remained significant after adjusting for these three SNPs mutually in the follow-up study of skin cancer controls (p-value=2.6×10^{-3}). Sulem et al. recently reported a pigmentation GWAS in the Icelandic population and showed a strong association between the variant rs1393350 in the *TYR* gene and eye color, freckles, and skin sensitivity to sun (Sulem *et al.*, 2007). The rs1393350 is in strong LD with a non-synonymous SNP rs1126809 (Arg402Gln) in the *TYR* gene (D′=1 and r^2=0.86), a common polymorphism of tyrosinase. Tyrosinase is a critical enzyme during melanosomal maturation and its high activity leads to the formation of eumelanosome (Jimbow *et al.*, 2000; Spritz, 1994). It has been reported that the *TYR* Arg402Gln was correlated with reduced pigmentation of the retina and iris resulting from low tyrosinase activity (Fukai *et al.*, 1995).

We identified that the three SNPs (rs352935, rs464349, and rs11648785) on chromosome 16 that showed significant associations with tanning ability in the initial GWAS and were confirmed in the follow-up study. Pooled p-values for rs352935, rs464349, and rs11648785 were 7.4×10^{-8}, 3.1×10^{-9}, and 2.7×10^{-9}, respectively. These SNPs are adjacent to the *MC1R* (melanocortin 1 receptor), a well-established pigmentation gene encoding a 317-amino acid 7-pass-transmembrane G protein-coupled receptor. As the rate-limiting step in the activation of the cAMP pathway in terms of melanin production, *MC1R* has been strongly associated with pigmentary phenotypes, especially with red-hair color phenotype. We had previously genotyped seven common *MC1R* variants among the NHS skin cancer controls (Han *et al.*, 2006). The analysis mutually adjusting for these 10 SNPs in the controls of the skin cancer study showed that the significant associations with tanning response after exposure to UV that we observed for three SNPs (rs352935, rs464349, and rs11648785) were eliminated by inclusion of the three *MC1R* red-hair color alleles (Arg151Cys, Arg160Trp, and Asp294His). This result suggests that the signals that we identified on chromosome 16 were explained by the functional variants in the *MC1R* gene, although the LD between the *MC1R* variants and surrounding highly significant SNPs was relatively low. Similar results were noted in the GWAS of hair color (Han *et al.*, 2008). We observed that the highly significant associations between the SNPs on chromosome 16 and hair color phenotype were eliminated after adjusting for functional *MC1R* variants.

There is some evidence that determinants of human pigmentation may act along different phenotypic axes. For example, alleles at the *MC1R* locus primarily determine presence or absence of red hair (Rees, 2004). Hence, we additionally evaluated the associations of 27 selected SNPs with tanning response after excluding individuals with red hair color. The association patterns were similar to those shown in the analyses including red haired individuals (Table S2).

One limitation of this study was the self-reported tanning information. Self-report has been shown to be an appropriate and widely-used method of assessing risk factors for skin cancer.

Test-retest reliability of collecting phenotypic factors from questionnaires is moderate to substantial, including skin color, tanning/burning tendency, and sunburn history(Branstrom *et al.*, 2002; Glanz *et al.*, 2003; Westerdahl *et al.*, 1996).

In our study of individuals of European ancestry, we focused on the most statistically significant associations from our GWAS, identifying and confirming the loci previously known as pigmentation genes, such as *MATP*, *IRF4*, *TYR*, *OCA2*, and *MC1R*. The strongest loci for tanning ability were the same as those for hair color. Because a subset of true associations would be weakly associated with outcome in any given GWAS, large-scale replication is necessary for confirmation, and some true associations may be missed if they are not carried forward into replication studies (Chanock *et al.*, 2007).

Materials and Methods

Nurses' Health Study (NHS)

The NHS was established in 1976, when 121,700 female U.S. 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. Biennial questionnaires with collection of exposure information on risk factors and (every 4 years since 1980) nutritional assessments have been collected prospectively. Along with exposures every 2 years, outcome data with appropriate follow-up of reported disease events, including melanoma and non-melanoma skin cancers, are collected. Overall, follow-up has been very high; after more than 20 years approximately 90% of participants continue to complete questionnaires. From May 1989 through September 1990, we collected blood samples from 32,826 participants in the NHS cohort. The information on tanning response after exposure to UV in childhood and adolescence was collected in the 1982 prospective questionnaire. The question was "as a child or adolescent, after repeated sun exposures, e.g., a two-week vacation outdoors, what kind of tan would you get?", and the multiple choices were "practically none, light tan, average tan, and deep tan".

Initial GWAS

We initially performed genotyping in a nested case-control study of postmenopausal invasive breast cancer within the NHS cohort (Tworoger *et al.*, 2007) using the Illumina HumanHap550 array, as part of the National Cancer Institute's Cancer Genetic Markers of Susceptibility (CGEMS) Project (Hunter *et al.*, 2007). We performed our initial genome-wide analysis on 528,173 SNPs in 2,287 women (Hunter *et al.*, 2007). All cases and controls were self-described as being of European ancestry. Four samples were excluded because of evidence of intercontinental admixture. Controlling for breast cancer case-control status made no material difference to the GWAS results.

Detailed methods related to the initial GWAS were published previously (Hunter *et al.*, 2007), including genotyping and quality control, initial assessment of sample completion rates, assessment of SNP call rates, concordance rate, deviation from Hardy Weinberg proportions in control DNA, and final sample selection and exclusion for association analysis.

The controls in the skin cancer nested case-control study within the NHS

The promising SNPs from the initial GWAS were further genotyped among 870 controls in the skin cancer nested case-control study within the NHS. The distribution of risk factors for skin cancer in the subcohort of those who donated blood samples was very similar to that in the overall cohort (Han *et al.*, 2006). 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 corresponding case was diagnosed.

Health Professional Follow-up Study (HPFS)

In 1986, 51,529 men from all 50 U.S. states in health professions (dentists, pharmacists, optometrists, osteopath physicians, podiatrists, and veterinarians) aged 40–75 answered a detailed mailed questionnaire, forming the basis of the study. Between 1993 and 1994, 18,159 study participantsprovided blood samples by overnight courier. The information on tanning response after exposure to UV was asked in the 1992 questionnaire.

The diabetes nested case-control studies within the NHS and HPFS

Two additional studies were used to genotype novel pigmentation loci: 3,750 samples from the nested case-control study of diabetes in the NHS and 2,405 samples from the nested case-control study of diabetes in the HPFS. All samples that we used were cases and controls from these two studies. Cases were incident cases of diabetes after blood collection, and controls were matched on age and history of cardiovascular disease. Controlling for case-control status made no material difference to the results.

There was no sample overlap among the initial GWAS, the skin cancer case-control study, and the two diabetes case-control studies. The study protocol was approved by the Institutional Review Board of Brigham and Women's Hospital and Harvard School of Public Health. Informed consent was obtained from all patients.

Statistical analysis

For the primary analysis of tanning response after exposure to UV we regressed an ordinal coding for tanning ability (1=deep tan; 2=average tan; 3=light tan; and 4=no tan) on an ordinal coding for genotype (0, 1, or 2 copies of SNP minor allele) separately for each SNP that passed quality control filters (Hunter *et al.*, 2007). Crude analyses that did not adjust for any other variables showed evidence of systematic bias (see Results and Discussion section). However, this bias was greatly reduced by adjusting for the four largest principal components of genetic variation. These principal components were calculated for all subjects on the basis of ca. 10,000 unlinked markers using the EIGENSTRAT software (Hunter *et al.*, 2007; Price *et al.*, 2006). The top four eigenvectors were chosen on the basis of significant ($p<0.05$) Tracy-Wisdom tests (Patterson *et al.*, 2006). Adjusting for up to the top 50 principal components did not further reduce the genomic control inflation factor λ_{GC}. We chose markers for genotyping in subsequent validation studies based on the p-values for association from the primary analysis. The regression parameter beta refers to the mean change in tanning ability scoring per copy of the SNP minor allele. Pooled analyses of multiple studies were conducted by merging data sets and including separate baseline parameters for each study.

Genotyping in follow-up studies

The 27 promising SNPs from the initial GWAS were genotyped in the skin cancer controls using TaqMan/BioTrove assays at the Dana Farber/Harvard Cancer Center Polymorphism Detection Core. A novel locus rs966321 on chromosome 1 was further genotyped in diabetes samples in the NHS and HPFS studies using the Taqman assay. Laboratory personnel were blinded to the case-control status, and 10% 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

OR
: odds ratio

CI
: confidence interval

UV
: ultraviolet

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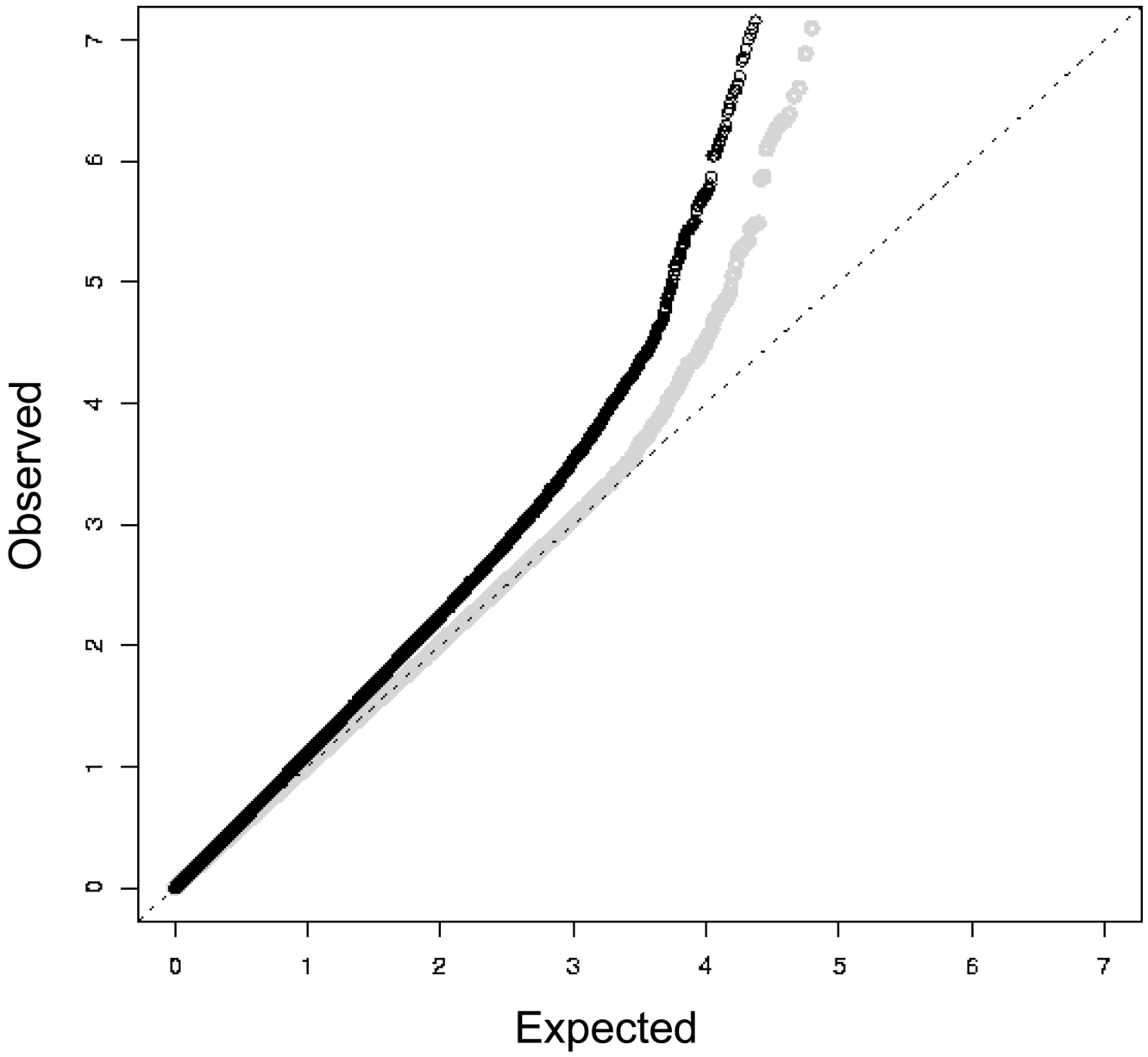

Figure 1.
Quantile-quantile plot of the -$\log_{10}$ p-values from an analysis of the initial GWAS that did not adjust for principal components of genetic variation (black dots) and an analysis that did adjust for the four largest principal components (graydots). P-values smaller than 10^{-8} are not plotted.

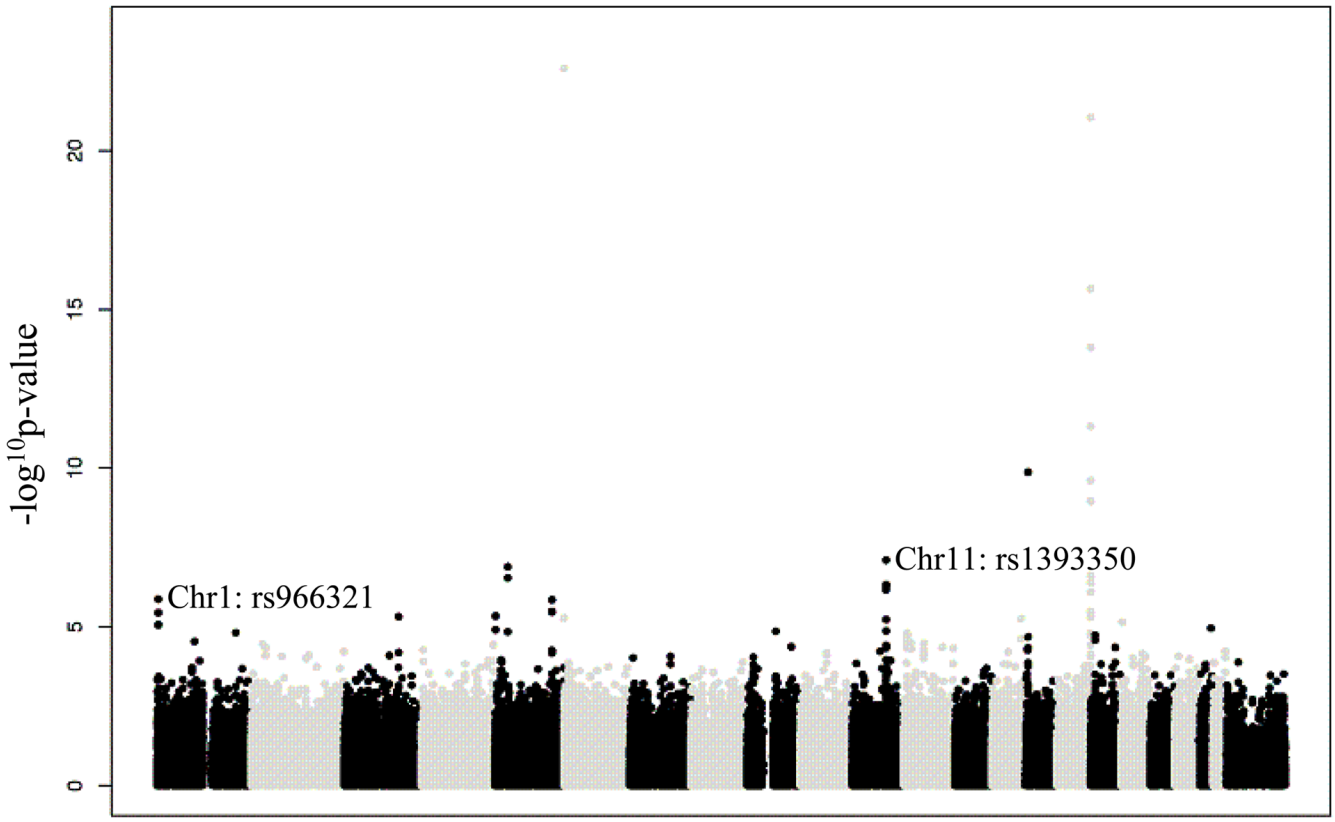

Figure 2.
−log10 p-values from the test of association with tanning response after exposure to UV in the initial GWAS, by position along chromosome. Only p-values smaller than 0.05 are plotted.

Table 1

Sixty SNPs with the smallest p-values of the 528,173 tested for association of tanning ability in the initial GWAS of in 2,287 women of European ancestry.

SNP	Chromosome	Location	Gene Neighborhood	WT/VT	MAF	beta	s.e.	p value	Presented in the GWAS of hair color*	Selected for replication
rs12203592	6	341321	IRF4	C/T	0.17	0.34	0.03	2.5E-23	Yes	
rs258322	16	88283404	MC1R	C/T	0.09	0.41	0.04	8.8E-22	Yes	
rs8049897	16	88551703	MC1R	G/A	0.15	0.30	0.04	5.8E-17	Yes	
rs4785763	16	88594437	MC1R	C/A	0.33	0.21	0.03	9.9E-15	Yes	
rs4238833	16	88578190	MC1R	T/G	0.37	0.19	0.03	3.7E-12	Yes	
rs12913832	15	26039213	HERC2/OCA2	G/A	0.25	−0.19	0.03	1.1E-10	Yes	
rs4408545	16	88571529	MC1R	T/C	0.50	0.16	0.03	2.0E-10	Yes	
rs164741	16	88219799	MC1R	C/T	0.30	0.17	0.03	9.4E-10	Yes	
rs1393350	11	88650694	TYR	G/A	0.26	0.16	0.03	8.0E-08	No	Yes
rs28777	5	33994716	MATP	A/C	0.03	−0.41	0.08	1.3E-07	Yes	
rs11648785	16	88612062	MC1R	C/T	0.32	−0.14	0.03	2.5E-07	No	Yes
rs35391	5	33991430	MATP	C/T	0.03	−0.42	0.08	2.9E-07	No	Yes
rs464349	16	88183752	MC1R	C/T	0.47	−0.13	0.03	4.1E-07	No	Yes
rs10831496	11	88197639	GRM5	A/G	0.31	−0.14	0.03	4.7E-07	No	Yes
rs10765198	11	88609422	TYR	T/C	0.28	0.15	0.03	4.7E-07	LD with rs1393350	
rs7119749	11	88154670	GRM5	G/A	0.31	−0.14	0.03	5.3E-07	LD with rs10831496	
rs10765770	11	88153284	GRM5	A/G	0.31	−0.14	0.03	6.1E-07	LD with rs10831496	
rs2169660	11	88179832	GRM5	G/A	0.31	−0.14	0.03	6.9E-07	LD with rs10831496	
rs7188458	16	88253985	MC1R	G/A	0.43	0.13	0.03	8.0E-07	Yes	
rs966321	1	4225577	LOC401937	A/C	0.48	−0.12	0.03	1.4E-06	No	Yes
rs32579	5	149191041	PPARGC1B	G/A	0.30	−0.14	0.03	1.4E-06	No	Yes
rs109075	5	149175116	PPARGC1B	T/C	0.30	−0.13	0.03	3.3E-06	LD with rs32579	
rs154659	16	88194838	MC1R	T/C	0.26	0.14	0.03	3.3E-06	No	Yes
rs109077	5	149176875	PPARGC1B	T/G	0.30	−0.13	0.03	3.5E-06	LD with rs32579	
rs2411738	1	4236741	LOC401937	A/G	0.50	−0.12	0.03	3.6E-06	LD with rs966321	
rs11133935	5	2244437		C/T	0.38	−0.12	0.03	4.6E-06	No	Yes
rs12493507	3	140744316	RBP1	C/T	0.11	0.19	0.04	4.8E-06	No	Yes
rs352935	16	88176081	MC1R	A/G	0.49	0.12	0.03	5.0E-06	No	Yes

SNP	Chromosome	Location	Gene Neighborhood	WT/VT	MAF	beta	s.e.	p value	Presented in the GWAS of hair color*	Selected for replication
rs12210050	6	420489	EXOC2	C/T	0.18	0.16	0.03	5.4E-06	No	Yes
rs17094273	14	96173560		G/A	0.10	0.20	0.04	5.5E-06	No	Yes
rs11018528	11	88570025	TYR	A/G	0.28	0.13	0.03	5.9E-06	LD with rs1393350	
rs9960018	18	3783080	DLGAP1	C/T	0.14	−0.17	0.04	7.2E-06	No	Yes
rs1908490	1	4222578	LOC401937	A/G	0.50	−0.11	0.03	8.5E-06	LD with rs966321	
rs6677984	1	4240660	LOC401937	G/A	0.49	−0.11	0.03	8.8E-06	LD with rs966321	
rs7279297	21	42100984	PRDM15	A/G	0.28	−0.13	0.03	1.1E-05	No	Yes
rs2897241	5	2255185		G/A	0.38	−0.12	0.03	1.3E-05	LD with rs11133935	
rs1847134	11	88644901	TYR	A/C	0.30	0.12	0.03	1.4E-05	LD with rs1393350	
rs1409937	9	75220958		A/G	0.47	0.11	0.03	1.4E-05	No	Yes
rs40132	5	33986460	MATP	T/C	0.42	−0.47	0.11	1.5E-05	No	Yes
rs12750212	1	206070491		G/A	0.10	−0.19	0.04	1.5E-05	No	Yes
rs1805761	12	8990800	M6PR	A/G	0.45	0.11	0.03	1.6E-05	No	Yes
rs2241039	16	88615938	MC1R	C/T	0.38	−0.12	0.03	1.6E-05	Yes	
rs1805733	12	8980539	M6PR	T/G	0.45	0.11	0.03	1.7E-05	LD with rs1805761	
rs10852800	17	11825241	ZNF18	C/T	0.19	0.14	0.03	1.8E-05	No	Yes
rs7204478	16	88322986	MC1R	C/T	0.44	0.11	0.03	1.9E-05	Yes	
rs1805721	12	9038836	KLRG1	C/T	0.45	0.11	0.03	2.0E-05	LD with rs1805761	
rs7495174	15	26017833	OCA2	A/G	0.08	−0.21	0.05	2.1E-05	Yes	
rs735408	14	96167406		G/A	0.09	0.19	0.05	2.4E-05	LD with rs17094273	
rs12449769	17	11809087	DNAH9	A/G	0.19	0.14	0.03	2.5E-05	LD with rs10852800	
rs1990236	17	11806187	DNAH9	G/A	0.19	0.14	0.03	2.7E-05	LD with rs10852800	
rs12821842	12	9000251	M6PR	G/A	0.45	0.11	0.03	2.7E-05	LD with rs1805761	
rs1805723	12	9033564	KLRG1	A/G	0.45	0.11	0.03	2.9E-05	LD with rs1805761	
rs1028889	1	98518442		C/T	0.26	0.12	0.03	2.9E-05	No	Yes
rs11054623	12	7626586		G/T	0.09	−0.19	0.04	3.2E-05	No	Yes
rs7974991	12	19082600	LOC90193	G/A	0.06	0.23	0.06	3.3E-05	No	Yes
rs7969151	12	52445544	LOC440100	G/A	0.21	0.13	0.03	3.3E-05	No	Yes
rs11170681	12	52451280	LOC440100	T/C	0.21	0.13	0.03	3.3E-05	LD with rs7969151	
rs1345151	2	30286837	YPEL5	G/A	0.20	0.14	0.03	3.5E-05	No	Yes

SNP	Chromosome	Location	Gene Neighborhood	WT/VT	MAF	beta	s.e.	p value	Presented in the GWAS of hair color*	Selected for replication
rs11931790	4	187715837	LOC285441	C/T	0.24	−0.13	0.03	3.7E-05	No	Yes
rs10830236	11	88540464	TYR	C/T	0.32	0.11	0.03	3.9E-05	No	Yes

*Detailed results for the SNPs indicated “yes” are presented in Table S1.

The p-values are based on primary association test adjusted for top four principal components of genetic variance.

The regression parameter beta refers to the mean change in tanning ability scoring per copy of the SNP minor allele.

Table 2

27 selected SNPs in the controls of the skin cancer study within the NHS and pooled data.

SNP	Chromosome	Location	Gene Neighborhood	WT/VT	in skin cancer controls			in pooled data		
					beta	s.e.	p value	beta	s.e.	p value
rs966321	1	4225577	LOC401937	A/C	−0.14	0.05	3.8E-03	−0.14	0.02	1.6E-09
rs1028889	1	98518442		C/T	0.03	0.05	0.57	0.10	0.03	1.4E-04
rs12750212	1	206070491		G/A	−0.02	0.08	0.78	−0.16	0.04	3.0E-05
rs1345151	2	30286837	YPEL5	G/A	0.05	0.06	0.33	0.12	0.03	2.0E-05
rs12493507	3	140744316	RBP1	C/T	0.05	0.07	0.45	0.16	0.04	1.0E-05
rs11931790	4	187715837	LOC285441	C/T	−0.04	0.05	0.48	−0.10	0.03	1.2E-04
rs11133935	5	2244437		C/T	−0.03	0.04	0.45	−0.09	0.02	1.3E-04
rs40132	5	33986460	MATP	T/C	−0.44	0.18	0.01	−0.53	0.09	1.5E-08
rs35391	5	33991430	MATP	C/T	−0.24	0.13	0.06	−0.44	0.07	3.2E-10
rs32579	5	149191041	PPARGC1B	G/A	−0.03	0.05	0.49	−0.11	0.02	3.6E-06
rs12210050	6	420489	EXOC2	C/T	0.28	0.06	5.7E-07	0.22	0.03	5.5E-14
rs1409937	9	75220958		A/G	0.06	0.04	0.16	0.10	0.02	2.0E-05
rs10831496	11	88197639	GRM5	A/G	−0.08	0.05	0.09	−0.14	0.02	4.7E-09
rs10830236	11	88540464	TYR	C/T	0.06	0.05	0.22	0.12	0.02	6.2E-07
rs1393350	11	88650694	TYR	G/A	0.16	0.05	7.3E-04	0.19	0.03	2.4E-13
rs11054623	12	7626586		G/T	0.04	0.08	0.61	−0.13	0.04	1.1E-03
rs1805761	12	8990800	M6PR	A/G	0.04	0.04	0.41	0.10	0.02	1.0E-05
rs7974991	12	19082600	LOC90193	G/A	−0.08	0.09	0.40	0.13	0.05	7.2E-03
rs7969151	12	52445544	LOC440100	G/A	0.10	0.05	0.06	0.13	0.03	1.5E-06
rs17094273	14	96173560		G/A	0.19	0.07	0.01	0.20	0.04	8.6E-08
rs352935	16	88176081	MC1R	A/G	0.11	0.04	0.01	0.12	0.02	7.4E-08
rs464349	16	88183752	MC1R	C/T	−0.12	0.04	4.9E-03	−0.14	0.02	3.1E-09
rs154659	16	88194838	MC1R	T/C	0.08	0.05	0.13	0.14	0.03	7.4E-08
rs11648785	16	88612062	MC1R	C/T	−0.10	0.05	0.04	−0.14	0.02	2.7E-09
rs10852800	17	11825241	ZNF18	C/T	0.04	0.06	0.47	0.12	0.03	7.0E-05
rs9960018	18	3783080	DLGAP1	C/T	−0.03	0.06	0.64	−0.15	0.03	1.0E-05
rs7279297	21	42100984	PRDM15	A/G	−0.03	0.05	0.49	−0.12	0.03	2.7E-06