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Predicting Phenotype from Genotype: Normal Pigmentation*

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Abstract

Genetic information in forensic studies is largely limited to CODIS data and the ability to match samples and assign them to an individual. However, there are circumstances, in which a given DNA sample does not match anyone in the CODIS database, and no other information about the donor is available. In this study, we determined 75 SNPs in 24 genes (previously implicated in human or animal pigmentation studies) for the analysis of single- and multi-locus associations with hair, skin, and eye color in 789 individuals of various ethnic backgrounds. Using multiple linear regression modeling, five SNPs in five genes were found to account for large proportions of pigmentation variation in hair, skin, and eyes in our across-population analyses. Thus, these models may be of predictive value to determine an individual's pigmentation type from a forensic sample, independent of ethnic origin.

Keywords

forensic science; genetics; human; pigmentation; QTL; phenotype

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Conflict of Interest Statement

Dr. Tony Frudakis is and Dr. Matthew Thomas was an employee and shareholder of a company, DNAPrint, which performs forensic DNA analysis.

FBI CODIS statistics showed that DNA forensic profiles increased exponentially from 2001–2006 (<http://www.fbi.gov/hq/lab/codis/clickmap.htm>). However, the DNA forensic profile hits increased linearly, suggesting that the discrepancy between unmatched DNA profiles and hits will continue to increase, as the CODIS DNA database increases. As a means of reducing the pool of suspects of an unmatched DNA profile, ancestry informative markers (AIMs) can indicate the ethnicity of an unknown sample. However, admixed samples are problematic, in that AIMs cannot categorize them into a particular ethnic group. Conceivably, an individual may have a majority of AIMs of one ethnic group, but depending on the amount of admixture, their physical appearance may be different than what might be expected.

While AIMs can enable inferences to be made about the ethnicity of an unknown sample, they do not enable identification of an individual based on physical characteristics within an ethnic group, hence as suggested by Tully (1), phenotype prediction based on genetic tests may be a useful tool in forensic analysis. In this study, we assayed genetic markers associated with pigmentation genes (some of which are also AIMs) to determine how much variation in human pigmentation they account for. These markers have been previously studied within specific ethnic groups. We demonstrate that in ethnically mixed populations, these same markers account for a significant fraction of the pigmentation phenotype.

There are two types of melanin, eumelanin (brown/black) and pheomelanin (yellow/red) that differ in sulfur content (2). Variation in human pigmentation results from differences in the type of melanin and amount of melanin synthesized in specialized vesicles (melanosomes) within pigment cells (melanocytes) and in the size, shape, and export of those melanosomes to the hair and skin (3). Although the rate of synthesis of melanin is much lower in the adult eye than in the skin and hair, additional background color is contained in the iris, making eye color a more complex trait. Genes previously implicated in mediating pigment variation include the melanocortin-1 receptor (*MC1R*) gene and a gene encoding its inhibitor, agouti signaling protein (*ASIP*); two genes associated with oculocutaneous albinism, *P(OCA2)* and *SLC45A2(OCA4)*, formerly named *MATP*; and most recently, *SLC24A5*, the human orthologue of the zebrafish *golden* gene.

Multiple polymorphisms in the *MC1R* gene have been linked to red hair and fair skin (4–8), and the *ASIP* gene, encoding an inhibitor of the *MC1R* ligand, α -MSH, has been linked to skin pigmentation (9–12). A wide range of OCA phenotypes has been noted for various mutations of *OCA2* (13) and *SLC45A2*, formerly named *MATP* (14–16). Similarly, a wide range of coat color phenotypes is seen in mice with various mutations in their respective orthologous genes (*p*, [17,18] and *Slc45a2*, formerly named *uw*: [19]). These observations suggested that variations in these genes may be associated with variation in the normal range of human pigmentation. Indeed, gene(s) associated with brown eyes and brown hair were found to map to chromosome 15q, with the *OCA2* gene as a prime candidate (20). Population studies have shown that specific polymorphisms in two genes associated with albinism, the *OCA2* gene and the *SLC45A2* gene, are strongly associated with variations in normal pigmentation of the hair (20–22), skin (22–25), and eyes (22,26–31).

In addition to its effects on pigmentation variation, the *SLC45A2* polymorphism rs16891982 (L374F) is a useful marker of population origin (32,33). Another marker of population origin that plays an important role in pigmentation, *SLC24A5* (or NCKX5, the human orthologue of the zebrafish *golden* gene), has been recently identified, with a coding polymorphism divergent between European/Caucasians and other human populations (32,34). *SLC24A5* has been shown to biologically affect pigmentation in zebrafish, *Danio rerio* (34), murine, and cultured human epidermal melanocytes (35).

Alleles of other pigmentation genes such as *tyrosinase-related protein 1 (TYRP1)* and *dopachrome tautomerase (DCT)* have been statistically associated with human iris pigmentation (28). In association with certain alleles of other genes, specific alleles of agouti signaling protein have also been associated with human iris color (28) as well as skin color (9–11).

Previous studies have focused on the effects of a limited number of genes on hair or skin color (4,5,24,34). The most comprehensive studies to date have focused on genome-wide-association of SNPs with pigmentation within specific populations (8,12,36,37). In this report, we set out to determine the markers predictive for human pigmentation, independent of ethnic origin. We assayed 75 polymorphisms in 24 genes that were previously implicated in human or animal pigmentation studies for the analysis of single- and multi-locus associations with hair, skin, and eye color in 789 individuals of various ethnic backgrounds. Multiple linear regression (MLR) modeling revealed that a surprisingly small number of markers account for large proportions of pigmentation variation in hair, skin, and eyes in our across-population analyses.

Materials and Methods

Participants

Informed consent was obtained from 791 participants recruited at the University of Arizona between the ages of 18 and 40 with no gray hair and at least 1 inch (measured from the roots) of un-dyed scalp hair. Participants in this study roughly mirror the ethnic composition of the student population. Phenotype data, hair samples, and buccal cell samples were collected from each participant following an Institutional Review Board-approved protocol. Participant's hair and eye color were independently scored by an investigator; participants also indicated other relevant information, such as tanning response and ethnicity. Buccal cell samples were collected using Catch-All Sample Collection Swabs (Epicentre, Madison, WI) and processed for DNA according to the manufacturer's protocol.

Melanin Analysis

Approximately 300 scalp hairs (1 cm at the base) were collected from each subject. Hair samples from 186 randomly selected participants were analyzed for both total melanin (combined amount of eumelanin and pheomelanin) content and the two subtypes of melanin, eumelanin and pheomelanin, following a previously published protocol (38,39). As not all genotypes were determined for all SNPs for all individuals, subsets of the 186 samples (54–185) were used in generating the multiple linear regression models discussed later.

Skin Reflectance

Skin reflectance was measured as others (40) have with a portable spectrophotometer (Mercury 1000, Datacolor International, Lawrenceville, NJ) fitted using a 15-mm aperture. This device measures in the visible light range of 400–700 nanometers, at intervals of 20 nm. Three independent reflectance measurements (measured as CIEL, L [lightness] scale of the International Committee on Illumination) of the inner aspect of the upper arm were recorded and averaged for each participant.

Eye Color

Eye color was measured by matching subjects' eye color to the Kolberg Iris Color Chart[®] (ocularistsupplies.com) and recorded. Measurements were binned into six different color categories based on another study (41) that correlated pigmentation content to color. Categories were binned one through six (1 = blue, 2 = yellow brown, 3 = green, 4 = packets

of brown + blue/green, 5 = brown, and 6 = dark brown/black), where bin 1 corresponded to the least amount of pigmentation, and 6 corresponded to the highest pigmentation.

Genotyping

Some SNPs were determined by sequencing the PCR products (Fig. 1). In this case, each PCR consisted of the final concentrations/quantities of the following: 1 ×PCR buffer, 1.5 mM MgCl₂, 10 pmol of each primer (forward and reverse; Table 1), 0.25 mM dNTPs, 1 U Taq, and ddH₂O to 20 μL. PCR amplification was performed using a PTC-200 Thermal Cycler (MJ Research, Water-town, MA). Thermal cycle program was as follows: 3 min at 95°C, 34 cycles of 30 sec. at each temperature setting (95°C, 55°C, and 72°C), and a final extension of 5 min at 72°C.

Sequencing was performed by the Genomic Analysis and Technology Core at the University of Arizona on a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). In addition, the first 287 DNA samples collected were genotyped at 75 SNPs in 24 genes implicated in melanin biosynthesis by DNA Print Genomics (Sarasota, FL) following a previously published protocol (27). The remaining 504 individuals were also genotyped by DNA Print Genomics (Sarasota, FL) for 37 of the 75 SNPs that were statistically significant.

SNP rs12913832 (HERC2) was genotyped using TaqMan assay C__30724404 (Applied Biosystems).

Mathematical Modeling

Statistical analysis was performed using SAS (version 9.1) and JMP IN (release 5.1) statistical analysis software (SAS Institute, Cary, NC). The pool of SNPs was reduced from 75 (Fig. 1; Table 2) to 40 (within 15 genes) by choosing SNPs that were statistically significant ($p < 0.05$, Table 2) by ANOVA ($n = 287$ samples). Finally, rs1426654 (*SLC24A5*), rs6058017 (*ASIP*), and rs12913832 (HERC2) were genotyped for all individuals ($n = 791$ samples). Two samples were dropped from the study (sample 102 and 352) because of bookkeeping inconsistencies. In accordance with standard statistical procedure, the natural log of the ratio of eumelanin-to-pheomelanin was used to normalize the data.

A total of 40 SNPs (Fig. 1; Table 2) were used to build MLR models of three SNPs. Initially, forward, reverse, and mixed stepwise regression methods were used to trim the number of SNPs. Different models were obtained based on the method used. To determine SNPs that were most significant in a three-SNP MLR model, we used SAS to generate all possible models of three SNPs from the pool of 40 significant SNPs. All models were then plotted using squared regression coefficient (R^2) in descending order of value, and inflections in the resulting curves were noted. To find the basis of these inflections, we plotted histograms using JMP IN that contained all SNPs that comprised all models up to the R^2 inflections (along the steepest initial slopes). In doing so, it became obvious which SNPs were predominantly responsible for the inflections. This method was performed in determining SNPs for each trait.

Results

Our primary aim was to develop a forensic DNA test predictive for pigmentation phenotype. Therefore, we analyzed the data across all populations of the study. The pool of SNPs was reduced from 75 to 40 SNPs by ANOVA ($p < 0.05$) (Fig. 1; Table 2). To determine the most likely SNPs for a given trait, all possible combinations of three-SNP models (40 choose 3) were generated, sorted by descending R^2 value, and graphed. The three most frequent SNPs found in the range from the highest R^2 model to the first inflection of the graph were used to

construct an MLR model for each trait. These models accounted for significant variation in each of the four measured traits: scalp-hair total melanin (76.3%), natural log of the ratio of eumelanin-to-pheomelanin (43.2%), skin reflectance (45.7%), and eye color (74.8%) (Table 3).

Scalp-Hair Total Melanin

The R^2 curve generated from the three-SNP models showed SNPs rs16891982 (SLC45A2), rs1426654 (SLC24A5), and rs12913832 (*HERC2*) to be the most frequent SNPs (310 or 3.1%) above the first inflection of the R^2 curve (Fig. 2A). Together these three SNPs yielded an R^2 of 76.3% ($n = 143$ samples).

Natural Log of the Ratio of Eumelanin-to-Pheomelanin

The R^2 curve generated from the three-SNP models showed SNPs rs16891982 (SLC45A2), rs1426654 (SLC24A5), and rs1805007 (*MC1R*) to be the most frequent SNPs (430 or 4.4%) above the first inflection of the R^2 curve (Fig. 2B). Together these three SNPs yielded an R^2 of 43.2% ($n = 162$ samples).

Skin Reflectance

The R^2 curve generated from the three-SNP models showed SNPs rs16891982 (SLC45A2), rs1426654 (SLC24A5), and rs2424984 (*ASIP*) to be the most frequent SNPs (750 or 7.6%) above the first inflection of the R^2 curve (Fig. 2C). Together these three SNPs yielded an R^2 value of 45.7% ($n = 447$ samples). An interaction term of rs2424984 (*ASIP*) and rs16891982 (*SLC45A2*) increased the R^2 value of the model by approximately 4% to 49.6% ($n = 447$ samples).

Eye Color

The R^2 curve generated from the three-SNP models showed SNP rs12913832 (*HERC2*) to be the most frequent SNP (710 or 7.2%) above the first inflection of the R^2 curve (Fig. 2D). rs12913832 yielded an R^2 value of 74.8% ($n = 484$ samples). The next two inflection points showed SNPs rs12913832 (*HERC2*), rs16891982 (SLC45A2), and rs1426654 (*SLC24A5*) to be the most frequent SNPs. Together these three SNPs yielded an R^2 value of 76.4% ($n = 353$ samples).

Discussion

Using MLR, we have shown that a large proportion of variation in hair, skin, and eye color across diverse human populations can be accounted for by a small number of SNPs. Starting from an initial candidate group of 75 SNPs, we found by ANOVA that 40 of these SNPs were significantly ($p < 0.05$) associated with hair pigmentation, skin reflectance, or eye color phenotype. Initially, we trimmed the number of SNPs by forward, backward, and/or a combination of both step-wise regression methods. However, each method yielded a different model. In an attempt to circumvent this problem, we modeled all possible combinations of significant SNPs. The R^2 values of the top models differed on average by <3–10 thousandths of a percent or smaller. The question arose as to which was the best model. To answer this question, we chose SNPs that were most frequent in the highest R^2 three-SNP models. This method ultimately trimmed the SNPs to five SNPs (three coding and two noncoding) in five genes that accounted for most of the variance (76.3% for hair total melanin; 43.2% for hair eumelanin-to-pheomelanin ratio; 45.7% for skin CIEL; and 74.8% for eye color).

Total Hair Melanin and Eumelanin-to-Pheomelanin Ratio

Previous studies have examined the overall color of hair in relationship to various genetic markers. Overall hair color is the result of at least two parameters: total melanin and the ratio of eumelanin-to-pheomelanin. These can be measured objectively by chemical analysis (2). We found that although both parameters are associated with SNPs from *SLC45A2* and *HERC2*, they differ in the third most significant genetic contributor (*SLC24A5* for total hair melanin and *MC1R* for the ratio of eumelanin-to-pheomelanin).

A high proportion of phenotypic variance of total hair melanin (76.3%) can be accounted for by three SNPs: rs1426654 (*SLC24A5*), rs16891982 (*SLC45A2*), and rs12913832 (*HERC2*) (Fig. 2A). While *SLC24A5* is an AIM, it plays an important role in pigmentation (34). And in studies carefully controlled for population stratification, the coding SNP rs1426654 is indeed a determinant for normal human pigmentation variation (42). Similarly, rs16891982 (*SLC45A2*) has been shown to be an AIM and has been shown to be associated with pigmentation variation in mice (19) and humans (22,32) in studies controlled for population stratification (42).

For the analysis of total hair melanin, SNP rs12913832 (*HERC2*) was the third most significant contributor in a three-SNP model. Although this SNP lies within the *HERC2* gene, it may be part of a promoter region for the adjacent *OCA2* gene (37,43–46). *OCA2* has been associated with albinism in diverse populations (13) and has also been associated with hair color by linkage analysis (20).

For the natural log of the ratio of eumelanin-to-pheomelanin, a high proportion of phenotypic variance (43.2%) can be accounted for by three SNPs. Two of these are in common with total hair melanin: rs1426654 (*SLC24A5*) and rs16891982 (*SLC45A2*) (Fig. 2B). SNP rs1805007 (*MC1R*) was the third most significant contributor in a three-SNP model. *MC1R* has been shown to be a major determinant in whether eumelanin or pheomelanin is produced (21,47). *MC1R* variants that decrease the protein's functionality have been shown to be associated with an increased incidence of red hair color in humans (4,5,48). Chemically, this translates to increased pheomelanin relative to eumelanin production.

Different studies have analyzed *MC1R* in different ways. Many analyzed specific populations to determine which SNPs were associated with variation in hair color, other studies focused on specific populations and on red hair. Moreover, the statistical analyses employed were different, and different variants have been studied. In this study, we analyzed for significance by (i) one-way ANOVA and (ii) a three-SNP model based on the frequency of contributors in the models with the highest R^2 values (Table 4).

Although we did not examine all known SNPs of *MC1R*, some of the SNPs we examined have been studied in relationship to red hair color. Valverde et al. (1995) found rs2228479 (V60L) in combination with other nonsynonymous *MC1R* SNPs in British and Irish to be associated with red hair color. In contrast, two studies (5,7) did not find an association of rs2228479 with red hair. Looking across populations of all hair colors, we did not find rs2228479 to be significant by ANOVA. In addition, Flanagan et al. (2000) and Branicki et al. (2007) found that rs1805007 (R151G) in combination with rs1805008 (R160W) to be associated with red hair color. Moreover, Sulem et al. (2007) found both of these SNPs to be associated with hair color in Icelandic and Dutch populations. In our three-SNP MLR model, rs1805007 (R151G) was the third most important genetic contributor for the ratio of pigmentation in hair color. We note that our analysis of the ratio of hair pigmentation did not focus on any particular hair color.

Skin Reflectance (CIEL)

A high proportion of phenotypic variance of skin reflectance (45.7%) can be accounted for by three SNPs: rs1426654 (*SLC24A5*), rs16891982 (*SLC45A2*), and rs2424984 (*ASIP*) (Fig. 2C). Studies have examined SNPs rs1426654 (*SLC24A5*) and rs16891982 (*SLC45A2*) and found both to be associated with normal human pigmentation within various ethnic groups and allele frequencies across various ethnic groups (24,32,34,42). Thus, rs1426654 (*SLC24A5*) and rs16891982 (*SLC45A2*) mediate pigmentation variation, and they are also AIMs. Lamason et al. (34) found SNP rs1426654 to be an AIM in determining European vs. non-European ethnic origin. Stokowski et al. (2007) controlled for population stratification and showed rs1426654 (*SLC24A5*) to be significantly associated with a dichotomously defined skin reflectance in South Asians. Although it is an AIM, it does not clearly distinguish between Europeans and Sri Lankans (32). In contrast, SNP rs16891982 (*SLC45A2*) does distinguish between Europeans and Sri Lankans (32).

The third most significant genetic contributor was SNP rs2424984 (*ASIP*). *ASIP* has been shown to be associated with skin pigmentation (12), namely for rs6058017 (9,10). Although we found rs6058017 to be significant by ANOVA, we did not find it to be a better predictor in skin reflectance than rs2424984. For both a single SNP and a three-SNP model, rs2424984 was a better predictor for skin reflectance.

Other studies have found *MC1R* (21,25,43,47), *OCA2*, and *DCT* (49) to be associated with normal skin pigmentation. Similarly, we found SNPs within the above genes to be associated by ANOVA with normal skin pigmentation. However, none were found to be significant contributors in a three-SNP MLR model. This does not imply that these genes are not important in pigmentation, it simply means that our method was unable to detect their significance. This may be attributed to a variety of factors such as sampling error because of small sample size, different populations studied, and/or different methods of measuring skin reflectance.

Eye Color

A high proportion of phenotypic variance of eye color (74.8%) can be accounted for by one SNP rs12913832 (*HERC2*) (Fig. 2D). Previous studies have examined eye color in relationship to various genetic markers. *OCA2* (8,20,26,28,29), *SLC45A2* (22), and *MC1R* (8,50) have been statistically associated with variation in eye color.

Duffy et al. (2007) found three SNPs within intron 1 of *OCA2* that when considered as a haplotype–diplotype explained about 74% of eye color variation. However, there are significant differences between our results and theirs. Among the differences were populations studied, SNPs genotyped, and binning of eye colors. The population they studied was Northern European, whereas we analyzed across various ethnic populations, and we genotyped additional SNPs within genes other than *OCA2*.

Most recently, genome-wide-association studies have shown that intronic SNPs of a gene 5' to *OCA2*, *HERC2*, have given the highest association with eye color (37,43,45). Studies suggest that *HERC2* contains a promoter region for *OCA2* (37,43,45,46,51). To date, SNP rs12913832 (*HERC2*) in intron 86 was shown to have the highest association and most likely causative SNP in determining eye color (45), explaining 68% of the variance between blue eye and brown eye color in an Anglo-Celtic population. Therefore, rs12913832 is likely the causative SNP for European/Caucasians. We note that rs12913832 varies in Caucasian/European populations (where eye color is varied), but only one allele is found in non-Caucasian/European populations (where most non-Caucasian/Europeans have brown/dark eye color). Regardless of whether rs12913832 is causative or an AIM, it is extremely predictive for eye color both within and across populations.

A three-SNP model of rs12913832 (*HERC2*), rs16891982 (*SLC45A2*), rs1426654 (*SLC24A5*) is only marginally better at explaining the variance (76%) than rs12913832 (*HERC2*) alone. Like, rs12913832 (*HERC2*), SNPs rs16891982 (*SLC45A2*), rs1426654 (*SLC24A5*) are AIMs that distinguish European/Caucasians from non-European/Caucasians. However, individually rs16891982 (*SLC45A2*) accounts for 38% of eye color variance, and rs1426654 (*SLC24A5*) accounts for 34% of eye color variance, significantly less than rs12913832 (*HERC2*) that accounts for 74% of the variance.

Statistical Interactions

Statistical interaction of SNPs rs12910433 (*OCA2*) and rs2228479 (*MC1R*) has been associated with skin reflectance in a Tibetan population (40). We did not find this interaction; however, we did find an interaction between SNPs rs16891982 (*SLC45A2*) and rs2424984 (*ASIP*) for the across-population skin reflectance MLR model. These differences may reflect our use of an ethnically diverse sample; thus, the statistical product of these two genes might be more predictive of skin reflectance across populations. Other interactions, such as between rs12913832 (*HERC2*) and rs1800407 (*OCA2*), may exist in explaining variation in eye color (45), but we cannot confirm this from our data.

Conclusion

We found five SNPs in five genes that were informative for normal human pigmentation (*SLC45A2*, *SLC24A5*, *MC1R*, *HERC2*, and *ASIP*). Three of these (*SLC45A2*, *SLC24A5*, and *MC1R*) were coding. Clearly, variations within these proteins can result in functional variation that contributes to the pigmentation variation. In contrast, the most significant SNPs found in *OCA2-HERC2* and *ASIP* were noncoding. We genotyped most of all known coding SNPs within these genes that showed allele frequency differences across populations, and none were as significantly correlated with pigmentation as the informative noncoding SNPs. This suggests that the regulation of the expression of *OCA2* and *ASIP* may underlie pigmentation variation. Although our results demonstrate that relatively few SNPs in a relatively few genes control a significant proportion of normal human pigmentation variation, it is certain that additional polymorphisms in these and other genes account for the remaining variation.

The polymorphisms that we report are in genes known to regulate pigmentation. Some or all of the variance in pigmentation explained by them may be because they are also AIMs. Nevertheless, they are predictive markers for normal human pigmentation variation across various ethnic backgrounds. We note that these models of human constitutive pigmentation phenotype have significant implications for forensic science. These results suggest that assays can be developed, independent of ethnicity, to predict hair, skin, and eye color from DNA samples. Preliminary analysis of an independent sample set ($n = 261$ samples) has validated the predictive utility of these models (Valenzuela et al., in preparation). In addition, recent genome-wide-association studies have uncovered a variety of other candidate SNPs that contribute to phenotypic variation in pigmentation. These include SNPs rs12896399 (*SLC24A4*), rs12203592 (*IRF4*), rs1540771 (*6p25.3*), and rs35264875 (*TPCN2*) (12,43,46). Future studies are needed to evaluate these and other SNPs as they are uncovered, to further refine these predictive models such that they can accurately predict the pigmentation phenotype of the donors of otherwise unknown forensic samples.

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Method for determining three-SNP MLR models

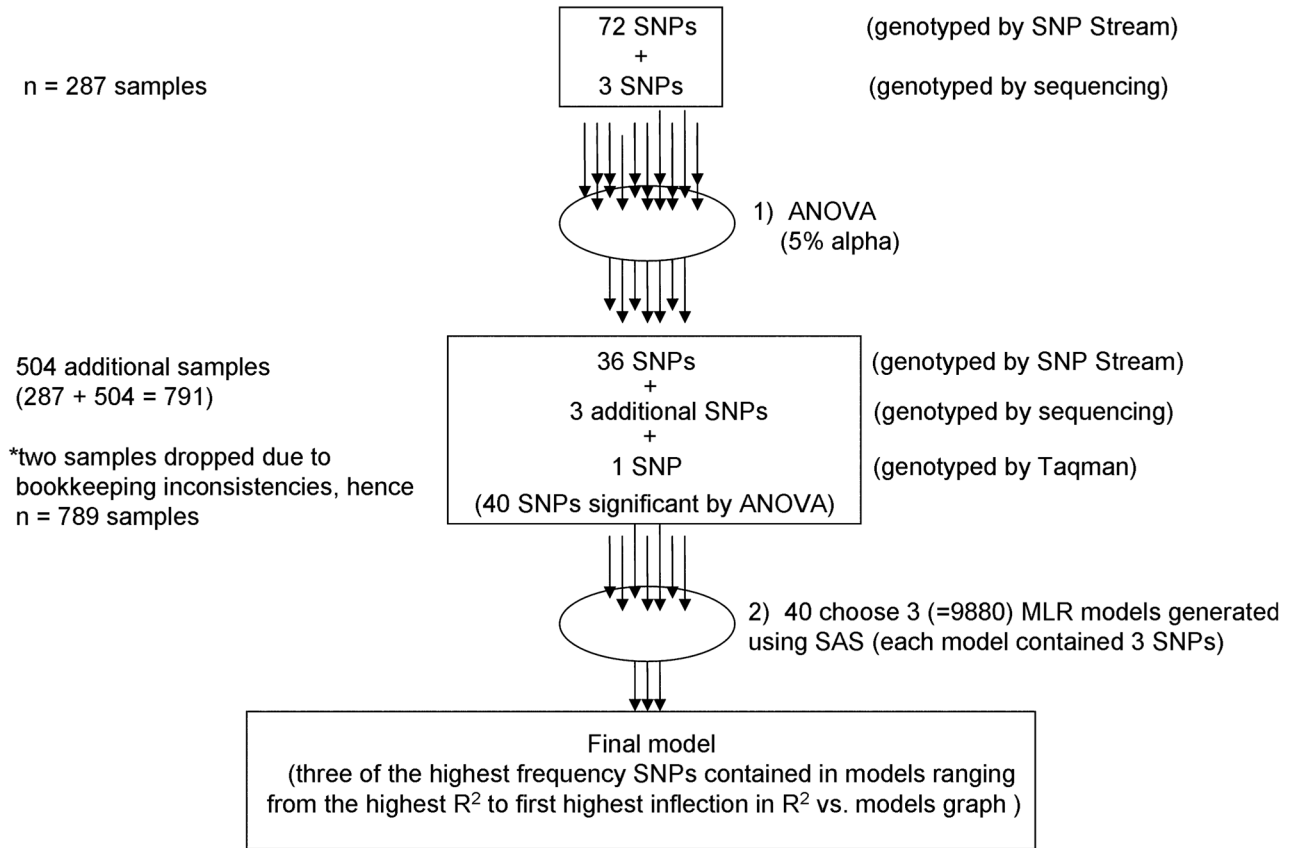
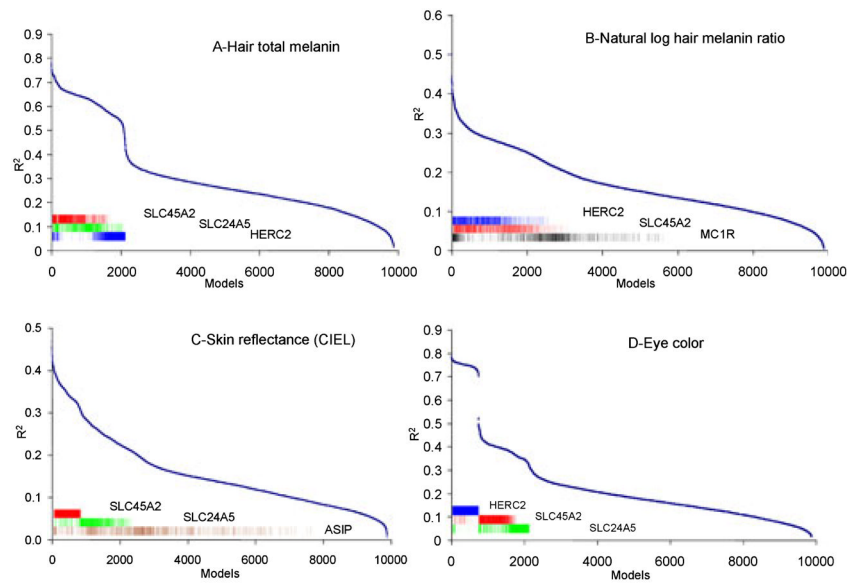


FIG. 1. Flow diagram of method for determining three-SNP multiple linear regression models.

**FIG. 2.**

Three-SNP multiple linear regression (MLR) models for four pigmentation traits across populations. In each panel, the horizontal-axis depicts all 9880 combinations of significant SNPs in a three-SNP MLR model (i.e., 40 choose 3). The vertical-axis is the R^2 value for each model. The three SNPs that occurred most frequently in the models with the highest R^2 values are indicated by the colored bar insets. Each model contained rs16891982 (SLC45A2) in red. (A) Hair total melanin's second and third SNPs were rs1426654 (SLC24A5) in green, and rs12913832 (HERC2) in blue, model R^2 value of 76.3%. (B) Natural log of hair melanin ratio's second and third SNPs were rs1426654 (SLC24A5) and rs1805007 (MC1R) in black, model R^2 value of 43.2%. (C) Skin reflectance's second and third SNPs were rs1426654 (SLC24A5) and rs2424984 (ASIP) in brown, model R^2 value of 45.7%. (D) Eye color's SNP was rs12913832 (HERC2) in blue, model R^2 value of 74.8%. The second and third SNPs rs16891982 (SLC45A2) and rs1426654 (SLC24A5) only marginally increased the model R^2 value to 76.4%.

TABLE 1

Primers used to amplify OCA2 exons 9 and 13 and sequences containing SNPs rs1426654 and rs6058017.

	Primer Name	Primer Sequence
rs1800401, and rs1800402 (<i>OCA2</i>)		
forward	MHB581	(5'-GGGCTGAATTGTCCATTTG-3')
reverse	MHB582	(5'-TCTCACGGATCTCAAGCCTC-3')
sequencing	MHB583	(5'-TGGCTGATACAGAGGGAGGT-3')
rs1800407 (<i>OCA2</i>)		
Forward	MHB593	(5'-GGCTCCCTGTTCTTAAAGTC-3')
Reverse	MHB594	(5'-TGAGAATGGAACCTGGAGCC-3')
Sequencing	MHB595	(5'-AAGGCTGCCTCTGTTCTACG-3')
rs1426654 (<i>SLC24A5</i>)		
Forward	MHB1755	(5'-TCATAAAGAAGCAAAACATTGGA-3')
Reverse	MHB1756	(5'-AGCACAGATGCCAAGGAGAT-3')
Sequencing	MHB1757	(5'-TGCCAATATCTCCCTTTGTG-3')
rs6058017 (<i>ASIP</i>)		
Forward	MHB1836	(5'-GGCTTCGATGAAGAAAGTGG-3')
Reverse	MHB1837	(5'-AGGGGATAGCCTCGTTCCTA-3')
Sequencing	MHB1838	(5'-ATGGGACTTCAGGGAGACCT-3')

TABLE 2

75 SNPs within 24 genes.

Gene	SNP	Total Hair Melanin	Skin Reflectance (CIEL)	Eye Color	Gene	SNP	Total Hair Melanin	Skin Reflectance (CIEL)	Eye Color	Natural Log of Ratio of Melanins		
										Total Hair Melanin	Skin Reflectance (CIEL)	Eye Color
<i>AP3D1</i>	rs2238600				<i>MC1R</i>	rs3212353				Natural Log of Ratio of Melanins		
<i>AP3D1</i>	rs4806830				<i>MC1R</i>	rs3212352				Natural Log of Ratio of Melanins		
<i>ASIP</i>	rs2424984	*	*	*	<i>MC1R</i>	rs3212351				Natural Log of Ratio of Melanins		
<i>ASIP</i>	rs6058017	*	*	*	<i>MLPH</i>	rs2292885	*			Natural Log of Ratio of Melanins		*
<i>ASIP</i>	rs2296151				<i>MLPH</i>	rs1343768				Natural Log of Ratio of Melanins		
<i>CYP2C8</i>	rs1341164				<i>MYO18A</i>	rs11080078				Natural Log of Ratio of Melanins		*
<i>CYP2D6</i>	rs1058174				<i>MYO5A</i>	rs1724630				Natural Log of Ratio of Melanins		*
<i>CYP4B1</i>	rs1572603				<i>MYO5A</i>	rs752864				Natural Log of Ratio of Melanins		*
<i>DCT</i>	rs1325611	*	*	*	<i>MYO5A</i>	rs2290332	*			Natural Log of Ratio of Melanins	*	*
<i>DCT</i>	rs1407995	*	*	*	<i>MYO7A</i>	rs2276289	*			Natural Log of Ratio of Melanins	*	*
<i>GPR143</i>	rs3044	*	*	*	<i>MYO7A</i>	rs3737454	*			Natural Log of Ratio of Melanins	*	*
<i>GPR143</i>	rs3810741				<i>MYO7A</i>	rs948962				Natural Log of Ratio of Melanins		
<i>GPR143</i>	rs2521667				<i>MYO7A</i>	rs1320703				Natural Log of Ratio of Melanins		
<i>HERC2</i>	rs12913832	*	*	*	<i>MYO7A</i>	rs2276288				Natural Log of Ratio of Melanins		*
<i>HPS1</i>	rs1804689				<i>OCA2</i>	rs1037208				Natural Log of Ratio of Melanins	*	*
<i>HPS1</i>	rs2296430				<i>OCA2</i>	rs10852218				Natural Log of Ratio of Melanins	*	*
<i>HPS1</i>	rs3830015				<i>OCA2</i>	rs11638265	*			Natural Log of Ratio of Melanins	*	*
<i>HPS3</i>	rs2689234				<i>OCA2</i>	rs1800404	*			Natural Log of Ratio of Melanins	*	*
<i>HPS4</i>	rs1894704				<i>OCA2</i>	rs1800407	*			Natural Log of Ratio of Melanins	*	*
<i>HPS4</i>	rs3752589			*	<i>OCA2</i>	rs1800410	*			Natural Log of Ratio of Melanins	*	*
<i>HPS4</i>	rs3752590			*	<i>OCA2</i>	rs1800411	*			Natural Log of Ratio of Melanins	*	*
<i>HPS4</i>	rs739289				<i>OCA2</i>	rs1800414	*			Natural Log of Ratio of Melanins	*	*
<i>HPS5</i>	rs2305564				<i>OCA2</i>	rs1900758	*			Natural Log of Ratio of Melanins	*	*
<i>HPS6</i>	rs4917959				<i>OCA2</i>	rs749846	*			Natural Log of Ratio of Melanins	*	*
<i>LYST</i>	rs3768051				<i>OCA2</i>	rs1800402				Natural Log of Ratio of Melanins		
<i>MAOA</i>	rs979605				<i>OCA2</i>	rs737051				Natural Log of Ratio of Melanins		
<i>MC1R</i>	rs1805007			*	<i>OCA2</i>	rs1800415				Natural Log of Ratio of Melanins	*	*

Gene	SNP	Natural Log of Ratio of Melanins			Gene	SNP	Natural Log of Ratio of Melanins		
		Total Hair Melanin	Skin Reflectance (CIEL)	Eye Color			Total Hair Melanin	Skin Reflectance (CIEL)	Eye Color
<i>MC1R</i>	rs1805008	*	*	*	<i>OCA2</i>	rs2305253			
<i>MC1R</i>	rs3212346	*			<i>OCA2</i>	rs1800401			
<i>MC1R</i>	rs3212355		*		<i>OCA2</i>	rs2044627			
<i>MC1R</i>	rs3212357	*	*	*	<i>SLC24A5</i>	rs1426654	*	*	*
<i>MC1R</i>	rs3212370				<i>SLC45A2</i>	rs16891982	*	*	*
<i>MC1R</i>	rs3212368				<i>SLC45A2</i>	rs2287949	*	*	*
<i>MC1R</i>	rs3212366				<i>SLC45A2</i>	rs26722	*	*	*
<i>MC1R</i>	rs3212363				<i>SLC45A2</i>	rs40132	*	*	*
<i>MC1R</i>	rs3212364				<i>TYRP1</i>	rs2733832	*	*	*
<i>MC1R</i>	rs2228479				<i>TYRP1</i>	rs683			
<i>MC1R</i>	rs1805005								

* Significant SNPs by ANOVA ($p < 0.05$) (40 SNPs within 16 genes) with respect to each of the four phenotypes for the first 287 samples. rs3212363 (*MC1R*) was genotyped for all individuals, it was not significant across all populations.

TABLE 3

Across populations R^2 values of individual SNPs, interaction terms, full model, and sample size of each ethnic group (self-reported).

	Individual R^2 Values % (sample size; p value)	Caucasian	African-American	Hispanic	South Asian	East Asian	Native American	Admixture	Not Listed
Scalp-hair total melanin									
rs16891982 (<i>SLC45A2</i>)	63.7 ($n=172$, $p=7.2 \times 10^{-38}$)	102	18	14	4	10	5	19	
rs1426654 (<i>SLC24A5</i>)	62.3 ($n=166$, $p=2.76 \times 10^{-35}$)	101	18	11	2	8	5	21	
rs12913832 (<i>HERC2</i>)	54.0 ($n=176$, $p=6.69 \times 10^{-30}$)	108	16	13	4	10	5	20	
Model	76.3 ($n=143$)	86	14	10	2	8	5	18	
In(eumelanin/pheomelanin)									
rs16891982 (<i>SLC45A2</i>)	24.7 ($n=172$, 3.88×10^{-11})	102	18	14	4	10	5	19	
rs12913832 (<i>HERC2</i>)	26.5 ($n=176$, $p=2.79 \times 10^{-12}$)	108	16	13	4	10	5	20	
rs1805007 (<i>MC1R</i>)	14.0 ($n=186$, $p=1.41 \times 10^{-7}$)	113	19	14	4	10	5	21	
Model	43.2 ($n=162$)	97	15	13	4	10	5	18	
Average skin reflectance									
rs16891982 (<i>SLC45A2</i>)	32.1 ($n=487$, $p=2.28 \times 10^{-41}$)	267	19	83	23	42	14	30	9
rs1426654 (<i>SLC24A5</i>)	23.9 ($n=528$, $p=7.09 \times 10^{-32}$)	296	20	88	22	41	15	33	13
rs2424984 (<i>ASIP</i>)	15.6 ($n=538$, $p=2.18 \times 10^{-20}$)	300	20	89	25	44	15	32	13
Model	45.7 ($n=447$)	244	19	74	20	39	14	28	9
Model+rs16891982* rs2424984	49.6 ($n=447$)								
Eye color									
rs12913832 (<i>HERC2</i>)	74.8 ($n=484$, $p=1.09 \times 10^{-144}$)	253	19	70	39	48	15	28	12
rs16891982 (<i>SLC45A2</i>)	38.4 ($n=397$, $p=3.55 \times 10^{-42}$)	191	21	56	35	47	13	24	10
rs1426654 (<i>SLC24A5</i>)	34.3 ($n=490$, $p=3.64 \times 10^{-45}$)	260	21	69	36	46	15	30	13
Model	76.4 ($n=353$)	171	16	49	32	43	13	20	9

TABLE 4

ANOVA results for MC1R SNPs with respect to hair total melanin and natural log of eumelanin-to-pheomelanin.

MC1R SNP	Total Melanin (From First 287 Individuals)	n	Ratio	Total Melanin (From Full Data Set)	n	Ratio
rs3212357	†	141	†	†	219	†
rs3212346	*	138	†	†	191	†
rs1805008	*	141	†	§	218	†
rs1805007	§	141	†	†	219	†
rs3212355	§	137	§	†	214	§
rs3212370	‡	141	‡			
rs3212368	‡	141	‡			
rs3212364	‡	141	‡			
rs3212352	‡	141	‡			
rs3212351	‡	141	‡			
rs3212366	§	137	§			
rs3212353	§	136	§			
rs1805005	§	124	§			
rs2228479	§	122	§			

* $p < 0.05$.† $p < 0.01$.

‡ No variation.

§ $p > 0.05$.