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Genome-wide association meta-analysis of individuals of European ancestry identifies new loci explaining a substantial fraction of hair color variation and heritability

Pirro G. Hysi [1,2]*, Ana Valdes [1, 3]*, Fan Liu[4, 6] *, Nicholas A. Furlotte [7], David M. Evans [8, 9], Veronique Bataille [1] , Alessia Visconti[1], Gibran Hemani [9], George McMahon [9], Susan M. Ring [9], George Davey Smith [9], David L. Duffy [10], Gu Zhu [10], Scott D. Gordon [10], Sarah E. Medland [10], Bochao D. Lin [11], Gonneke Willemsen [11], JoukeJan Hottenga [11], Dragana Vuckovic [12. 13], Giorgia Girotto [12, 13], Ilaria Gandin [13], Cinzia Sala [14], Maria Pina Concas [15], Marco Brumat [13], Paolo Gasparini [12,13], Daniela Toniolo [14], Massimiliano Cocca [13], Antonietta Robino [15], Seyhan Yazar [16], Alex W. Hewitt [16, 17, 18] , Yan Chen[4,5], Changqing Zeng[4], Andre G. Uitterlinden[19,20], M. Arfan Ikram[20], Merel A. Hamer[21], Cornelia M. van Duijn[20], Tamar Nijsten[21], David A. Mackey [16], Mario Falchi [1], Dorret I. Boomsma [11],Nicholas G. Martin [10], The International Visible Trait Genetics Consortium, David A. Hinds[7], Manfred Kayser [6,#,†,] and Timothy D. Spector [1,#,†]

Affiliations:

1 King's College London Department of Twins Research and Genetic Epidemiology, London, United Kingdom
2 Department of Ophthalmology, Kings College London, London, United Kingdom
3 Division of Rheumatology, Orthopaedics and Dermatology, School of Medicine, University of Nottingham, Nottingham, United Kingdom
4 Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, PR China
5 University of Chinese Academy of Sciences, Beijing, PR China
6 Department of Genetic Identification, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands
7 23andMe, Inc., Mountain View, California, USA
8 University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Queensland, Australia
9 MRC Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom
10 QIMR Berghofer Medical Research Institute, Brisbane, Australia
11 Netherlands Twin Register, Department of Biological Psychology, Vrije Universiteit, Amsterdam, the Netherlands
12 Experimental Genetics Division, Sidra Medical and Research Centre, Doha, Qatar
13 Department of Medical Sciences, University of Trieste, 34100 Trieste, Italy
14 Division of Genetics of Common Disorders, S. Raffaele Scientific Institute, 20132, Milan, Italy
15 Institute for Maternal and Child Health IRCCS "Burlo Garofolo", 34100 Trieste, Italy
16 Centre for Ophthalmology and Visual Science, University of Western Australia, Lions Eye Institute, Perth, Western Australia, Australia
17 Centre for Eye Research Australia, University of Melbourne, Department of Ophthalmology, Royal Victorian Eye and Ear Hospital, Melbourne, Australia
18 School of Medicine, Menzies Research Institute Tasmania, University of Tasmania, Hobart, Australia
19 Department of Internal Medicine, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands
20 Department of Epidemiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands
21 Department of Dermatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands
*,# these authors, respectively, contributed equally to this work
† To whom correspondence shall be addressed

Hair color is one of the most recognizable visual traits in European populations and is under strong genetic control. Here we report the results of a genome-wide association study meta-analysis of almost 300,000 participants of European descent. We identified 123 autosomal and one X-chromosome loci significantly associated with hair color; all but 13 are novel. Collectively, SNPs associated with hair color within these loci explain 34.6% of red hair, 24.8% of blond hair and 26.1% of black hair heritability in the study populations. These results confirm the polygenic nature of complex phenotypes and improve our understanding of melanin pigment metabolism in humans.

Human pigmentation refers to coloration of external tissues due to variations in quantity, ratio and distribution of the two main types of the pigment melanin: eumelanin and pheomelanin[1]. Most melanin is produced by melanosomes[2,3], large organelles specialized in melanin synthesis and transportation located mainly in the epidermis, hair and iris as well as the central nervous system. Early humans had a darkly pigmented skin[4,5] which protected against high Ultraviolet radiation (UVR) and its consequences such as skin cancer[6] and folate depletion[7]. European and Asian populations evolved to lighter skin pigmentation[8,9], as they migrated towards northern latitudes with lower UVR[4]. The lighter pigmentation maximizes UVR absorption needed to maintain adequate vitamin D levels. In Europeans, pigmentation of skin, hair and or eyes has characteristic geographic distributions because of natural selection[10] and perhaps genetic drift; a role for sexual selection has been debated [11-13].

Hair color is one of the most prominent traits in humans. Twin studies suggest that up to 97% of variation in hair color may be explained by heritable factors[14] and genome-wide association studies (GWAS) [15-20] have identified several chromosomal regions associated with hair color and related pigmentation traits[21]. Except for red hair, known variants have a relatively low predictive value[22] and the heritability gap remains relatively large[14] which suggests that many hair color genes, remain undiscovered.

Here we report the results of a meta-analysis of two GWAS carried out in two large discovery cohort studies: 157,653 research participants from the 23andMe, Inc. customer base[18] and 133,238 individuals from the UK Biobank (UKBB). Participants in both studies self-reported the natural color of their hair in adulthood (Supplementary Figure 1 and Supplementary Methods). For the purpose of this work, each hair color category collected (black, dark brown, light brown, red and blond) was assigned numerical values ranging from lowest (blond) to highest (black). These codes were used as the outcome variable in linear regression based GWAS analyses. To minimize population admixture and stratification, the analyses were restricted to individuals of European ancestry (Supplementary Figures 2 and 3) and adjusted for the first ten principal components (PCs) of the genotype matrix, as well as age and sex.

The analyses confirmed a strong association between hair color and PCs, especially in the less ethnically homogeneous 23andMe dataset, which includes participants of more varied European origin, in line with the known North-South cline in hair color variation and other regional differences in hair color across Europe[12] (Supplementary Table 1). The strongest associations in both groups were with sex (Table 1). Women were more likely to report blond (OR=1.20 and OR=1.29 in the 23andMe and UKBB participants, respectively), or red hair (OR=1.72 and OR=1.40, respectively) than any other color and three to five times less likely to report black hair (OR=0.35 and OR=0.20, respectively) compared to men.

Genomic inflation factors [23] (λ_{GC}) from the 23andMe and the UKBB GWAS were 1.147 and 1.146, respectively, in line with expectations of high power to detect large polygenic effects in these large samples[24] (Supplementary Figure 4). Meta-analyzed GWAS results reached conventional genome-wide

significance ($p<5x10^{-08}$) in many regions, primarily clustering around 123 distinct autosomal genomic SNPs and one X-chromosomal locus (Figure 1, Supplementary Table 2), mostly new (**Table 2**). In line with power expectations (Supplementary Figure 5), 75 of these regions were genome-wide significant in at least one of the two cohorts and always at least nominally significant ($p<0.01$) in the other.

Previously known pigmentation loci were all strongly associated in the meta-analysis results: *HERC2* (rs12913832), *IRF4* (rs12203592), *MC1R* (rs1805007), as well as others, showed some of the strongest statistical evidence for association ever published for human complex traits. Strong associations were found for genes whose mutations reportedly cause impairment of pigmentation such as Waardenburg (*EDNRB*, rs1279403, $p<10^{-100}$; *MITF*, rs9823839, $p<10^{-100}$), Hermansky-Pudlak (*HPS5*, rs201668750, $p=4.68x10^{-11}$), Trichomegaly (*FGF5* rs7681907, $p=5.684x10^{-25}$) or Ablepharon-Macrostomia (*TWIST2*, rs11684254, $p=1.233x10^{-20}$) Syndromes. Many polymorphisms significantly ($p<5x10^{-08}$) associated with hair color in our meta-analysis had existing entries in the GWAS Catalog[21]. In previous publications, they were associated to several phenotypes, including most known pigmentation loci (Supplementary Table 3).

Among the associated loci, some of the strongest effects were observed for two solute carrier 45A family members (*SLC45A1,* rs80293268*, $p<10^{-100}$* and the *SLC45A2* gene, rs16891982, $p<10^{-100}$); polymorphisms near a third solute carrier gene were also significantly associated with the trait (rs60086398 upstream of *SLC7A1*, $p=4.93x10^{-08}$). In addition, forkhead box family genes (*FOXO6,* rs3856254, $p=4.0x10^{-09}$ and *FOXE1*, rs3021523, $p=4.23x10^{-23}$) and sex determining region Y (SRY)-box genes (*SOX5* rs9971729, $p=8.8x10^{-17}$ and *SOX6,* rs1531903*,* $p=9.1x10^{-16}$) were among those highlighted in our results. An additional locus, located on chromosome X, on the second intron of the collagen type IV alpha 6 gene was also significantly associated (*COL46A*, rs1266744, $p=5.03x10^{-12}$). Chromosome Y information was not analyzed. Interestingly, given the observed strong association of hair color with sex, there was no particular difference in effect sizes observed for these loci among men and women in either cohort (Supplementary Table 4, Supplementary Figure 6); only one SNPs significantly associated with hair color in the meta-analysis showed significant ($p=1.6x10^{-08}$) interaction with sex in the 23andMe (Supplementary Table 5), but much weaker interaction in the UK Biobank cohort ($p=0.04$). As reported before[10], some hair color genes are subject to significant natural selection (Supplementary Table 6); SNPs associated with hair color in our meta-analysis, tended to have lower selection score centiles and higher than average evidence for natural selection within European populations ($p=0.04$) and compared to Africans (Supplementary Figure 7).

To further validate the results and to introduce a testing dataset, we collected GWAS summary statistics from 10 additional cohorts with 27,865 European participants from International Visible Trait Genetics (VisiGen) Consortium[25] and meta-analyzed them. For 114 of the 123 autosomal loci highlighted by the discovery GWAS meta-analysis, the direction of the association was the same as observed in the meta-analysis; despite the lower statistical power of the replication due to smaller sample sizes, most leading SNP loci from the discovery meta-analysis (75 of the 123 autosomal regions) replicated at least at a nominal level and the same direction of association ($p<0.05$); for 35 of these loci the association was stronger even after correction for multiple testing (Supplementary Table 2).

Next, we assessed the potential relationship of the most associated polymorphisms and expression of the genes nearest to them. In line with most previous GWAS[26], the majority of these polymorphic loci had eQTL effects in several tissues. The strongest associations were observed with transcript of the

CBWD1 (rs478882, p=1.30x10^{-30}), *PPM1A* (rs7154748, p=3.30x10^{-14}) and *RALY* genes (rs6059655 being associated with *ASIP* gene expression, p=6.0x10^{-09}) in sun-exposed skin tissues (Supplementary Table 7).

As expected, genes showing the strongest association in the meta-analysis were significantly enriched for several Gene Ontology entries, especially pigmentation, melanin biosynthetic and metabolic processes, etc. (***Figure 2***, Supplementary Table 8).

A conditional analysis of the discovery cohorts identified 258 SNPs independently associated with hair color (Supplementary table 9). These SNPs explain overall 20.68% of the hair color heritability (using ordinal categories) and 34.58% (SE=3.64%) of the population liability scale[27] heritability for red hair (vs. any other color, assuming population prevalence is as in the UKBB at 0.047), 24.80% for blond hair (SE=2.49%, assuming a prevalence of 0.11) and 26.12% (SE=3.11%) of the black hair heritability (prevalence 0.046, Table 3).

Finally, we modelled hair color prediction in two cohorts (QIMR N=7,283 and RS N=7,724) using the 258 independently associated SNPs from the discovery GWAS meta-analysis (Supplementary Table 9) together with previously reported SNP predictors for hair color from the HIrisPlex System[28]. We split the data into model building (80%) and validation (20%) sets to assure that marker discovery, model building and model validation were independently executed. In both cohorts, prediction accuracies were high for black (QIMR AUC=0.91, RS AUC=0.81) and red (0.87 and 0.84) hair colors, but lower for blond (0.79 and 0.74) and brown (0.76 and 0.64; Supplementary Table 10, Supplementary Figure 8). Using the same datasets, these new models outperformed the previous HIrisPlex model[22] (QIMR/RS black 0.82 vs 0.77, red 0.87 vs. 0.83, blond 0.67 vs. 0.65, brown 0.66 vs. 0.57, Supplementary Table 10).

Our work identified over a hundred new genetic loci involved in hair pigmentation in Europeans and raises interesting questions. First, the observation of higher prevalence of lighter hair colors among women (Supplementary Figure 9), follows previous findings based on objective quantitative measurement of hair color[29,30] suggesting that sex is truly associated with hair color, independent of socially driven self-reporting bias. Second, although hair pigmentation spans a spectrum from very bright (blond) to very dark (black), the genetic mechanisms don't always follow this linear scale, as red hair color often has unique predisposing genetic factors [16,17]. However, our results explain even higher portions of heritability than before[14] for all hair colors and not just for the extremes of the light-dark hair color spectrum. Third, hair color is a trait that follows special distribution patterns of distribution, therefore is prone to issues of population structure bias that may be controlled in several ways. A comparison of different methodologies (Supplementary Figure 10) shows that our approach is roughly equivalent with others. Fourth, annotation of genetic regions based on physical distances and association probability most likely underestimates the number of regions involved in hair pigmentation. For example, the involvement of *OCA2* and *HERC2* genes in human pigmentation is not simply due to linkage disequilibrium[31], yet because of their proximity, both loci in our study were assigned to the same association region. This would, however, not affect the conditional analysis at a marker level, which discriminates separate effects.

In conclusion, this large GWAS meta-analysis has improved our knowledge on the genetic controls of human hair and pigmentation by bringing the number of known genes into the hundreds. The newly identified genetic loci explain substantial portions of the hair color phenotypic variability and will guide future research into better understanding the functional mechanisms linking these genes to pigmentation variation. Our findings are also useful in the future for both the better molecular

understanding of human pigmentation including their DNA-based prediction as relevant in forensic and anthropological applications, and the diseases that result from biological impairment of pigmentation including the development of treatment strategies.

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AUTHOR CONTRIBUTION

PGH, AV, FL jointly wrote the manuscript, coordinated meta-analyses and prediction modelling; NAF, DME, VB, AV, GH, GM, SMR, DLD, GZ, SDG, SEM, BDL, GW, JJH, DV, GG, IG, CS, APC, MB, DT, MC, AR, SY, AWH, YC, CZ, AGU, MAH, TN, MF, DAH each conducted part of the analyses described in this work; GDS, PG, CMvD, MAI, DAM, DIB, NGM, MF contributed populations samples and data used for analyses; MK and TDS jointly coordinated the work and participated in manuscript preparation.

COMPETING FINANCIAL INTERESTS

NF and DAH are employees of the 23andMe Inc. consumer genetics company.

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Figure Legends

Figure 1. Manhattan plot of the inverse variance meta-analysis for association with hair color of the 23andMe and UKBB cohorts (meta-analysis N=290,891). The unadjusted significance of association (y-axis) for each SNP on different chromosomes is shown in alternating navy and green along the x-axis with polymorphisms reaching significance at GWAS level ($p<5x10^{-08}$) depicted in red. The values on the y-axis were truncated at $p=10^{-500}$.

Figure 2. Gene Ontology Biological Processes annotations for genes adjacent to the SNPs showing the strongest associations with hair color via GWAS meta-analysis in the 23andMe and UKBB cohorts.

Tables

Table 1. Effect of sex on the hair color phenotypes in the 23andMe (N=157,653 independent participants) and UK Biobank (N=133,238 independent participants) cohorts.

23andMe	Odds Ratio	Standard Error	95% Confidence Interval	
			low	upper
Blond (all)	1.202	0.024	1.174	1.230
Red	1.721	0.014	1.675	1.768
Light Brown	1.116	0.013	1.088	1.145
Dark Brown	0.663	0.011	0.650	0.677
Black	0.348	0.030	0.329	0.369

UKBB	Odds Ratio	Standard Error	95% Confidence Interval	
			low	upper
Blond	1.285	0.018	1.241	1.330
Red	1.395	0.026	1.325	1.469
Light Brown	1.101	0.011	1.077	1.125
Dark Brown	0.993	0.011	0.971	1.015
Black	0.195	0.033	0.182	0.208

Table 2. A selection of genes newly associated with hair color. The selection was based on the strength of their effect, which is defined as the standardized linear regression coefficient. Results are given for the UK Biobank, 23andMe, their meta-analysis as well as the meta-analysis results from the VisiGen Consortium. These results were generated linear models and effect sizes (Beta) are given in SD units. The A, C, T and G under the "Reference Allele field" denote the nucleotide of the allele for which the effect size and allele frequencies are reported. Frequencies are given for the reference allele and are the average of observed frequencies in the 23andMe and UK Biobank. Associations with p-values of less than 10-100 are reported as "$p<10^{-100}$".

						UK Biobank				23andMe				Meta-analysis		
Chr	Pos(Build37)	SNP ID	Ref. Allele	Freq.	Nearest Gene	N	Beta	SE	p-value	N	Beta	SE	p-value	Beta	SE	p-value
1	8207579	rs80293268	G	0.047	*SLC45A1*	132221	0.194	0.009	1.54E-97	157651	0.157	0.009	1.29E-67	0.175	0.007	<E-100
1	205181062	rs2369633	T	0.089	*DSTYK*	132887	-0.071	0.007	9.20E-26	157651	-0.077	0.006	3.15E-38	-0.075	0.005	3.44E-62
2	28613302	rs71443018	G	0.039	*FOSL2*	126428	0.133	0.01	2.14E-39	157651	0.148	0.012	4.18E-33	0.139	0.008	1.36E-70
9	126808006	rs58979150	T	0.108	*LHX2*	132883	0.089	0.006	1.03E-44	157651	0.083	0.005	9.93E-53	0.086	0.004	1.40E-95
13	78391757	rs1279403	T	0.406	*EDNRB*	133238	-0.086	0.004	<E-100	157651	-0.074	0.004	4.57E-95	-0.08	0.003	<E-100
15	48426484	rs1426654	G	0.021	*SHC4*	133238	0.188	0.069	0.006	157651	0.289	0.03	2.12E-21	0.273	0.028	1.24E-22
17	39551099	rs117612447	T	0.029	*KRT31*	133238	0.063	0.011	2.95E-08	157651	0.064	0.011	2.09E-09	0.063	0.008	3.29E-16
20	52661068	rs73132911	T	0.046	*BCAS1*	132836	0.089	0.009	6.78E-22	157651	0.046	0.008	2.54E-09	0.064	0.006	5.85E-27

Table 3. Phenotypic variance explained by the identified autosomal loci significantly associated with hair color. The current estimates are given as the ratio of the genetic variance, V(G), over the phenotypic variance (Vp) and scaled over the population prevalence, V(G)/Vp_L, (estimated in the UKBB cohort, N=133,238), on the right. The estimates of genetic variance explained by known SNPs prior to this study were taken from previous publications. The phenotypes in this table were compared with all other hair colors. Since 80% of the participants reported some shade of brown hair color (dark or light), the heritabilities for these two phenotypes were considered baseline and were not calculated.

	Current heritability estimates					Previous estimates	
Phenotype	V(G)/Vp	SE	V(G)/Vp_L	SE	Prevalence	V(G)/Vp	SE
Blond	0.094	0.009	0.248	0.025	0.113	0.058	0.022
Red	0.074	0.008	0.346	0.036	0.046	0.069	0.069
Black	0.056	0.007	0.261	0.031	0.047	0.005	0.005

ONLINE METHODS

DATA AVAILABILITY

This work used data from two primary sources. The original datasets can be accessed as follows:
For UK Biobank data, through the UK Biobank Access management, as specified here: http://www.ukbiobank.ac.uk/register-apply/ . The hair color data accession codes are 1747.0.0, 1747.1.0 and 1747.2.0. The participants age UK Biobank accession code is 21022, for sex 31.0.0 and the pre-computed principal components used here 22009.0.1 through 22009.0.10 .

For the 23andMe participants requests for summary statistics access can be made at https://researchers.23andme.org/collaborations. There are no accession codes available.

For the TwinsUK datasets access can be asked through http://www.twinsuk.ac.uk/data-access/ and access to the secondary source of data through the corresponding authors.

Online Supplementary Methods

PARTICIPANTS, GENOTYPING AND PHENOTYPING

The 23andMe Cohort. All research participants were drawn from the customer base of 23andMe, Inc., a consumer genetics company. This cohort has been described in detail previously[32]. All participants included in the analyses provided informed consent and answered surveys online according to our human subjects protocol, which was reviewed and approved by Ethical & Independent Review Services, a private institutional review board (http://www.eandireview.com). Hair color phenotypes were used to create an ordinal trait with values: 0-light blond, 1-dark bond, 2- red, 3-light brown, 4-dark brown and 5-black. DNA extraction and genotyping were performed on saliva samples by CLIA-certified and CAP-accredited clinical laboratories of Laboratory Corporation of America. Samples were genotyped on one of four genotyping platforms. The V1 and V2 platforms were variants of the Illumina HumanHap550+ BeadChip., including about 25,000 custom SNPs selected by 23andMe, with a total of about 560,000 SNPs. The V3 platform was based on the Illumina OmniExpress+ BeadChip., with custom content to improve the overlap with our V2 array, with a total of about 950,000 SNPs. The V4 platform in current use is a fully custom array, including a lower redundancy subset of V2 and V3 SNPs. with additional coverage of lower-frequency coding variation, and about 570,000 SNPs. Samples that failed to reach 98.5% call rate were re-analyzed. For the GWAS only participants who have >97% European ancestry, as determined through an analysis of local ancestry, were included. For the purposes of ethnic categorization, an algorithm first partitioned phased genomic data into short windows of about 100 SNPs and used a support vector machine (SVM) to classify individual haplotypes into one of 31 reference populations. The SVM classifications then fed into a hidden Markov model (HMM) that accounts for switch errors and incorrect assignments, and gives probabilities for each reference population in each window. The reference population data are derived from public datasets (the Human Genome Diversity Project, HapMap, and 1000 Genomes), as well as 23andMe customers who have reported having four grandparents from the same country. A maximal set of unrelated individuals was chosen for each analysis using a segmental identity-by-descent (IBD) estimation algorithm[33]. Individuals were defined as related if they shared more than 700 cM IBD, including regions where the two individuals share either one or both genomic segments identical-by-descent. This level of relatedness corresponds approximately to the minimal expected sharing between first cousins in an outbred population.

Participant genotype data were imputed against the September 2013 release of 1000 Genomes Phase1 reference haplotypes, phased with ShapeIt2[34]. We phased and imputed data for each genotyping platform separately. We phased using an internally developed phasing tool which implements the Beagle haplotype graph-based phasing algorithm[35], modified to separate the haplotype graph construction and phasing steps.

SNPs with Hardy-Weinberg equilibrium P<10−20, call rate < 95%, or with large allele frequency discrepancies compared to European 1000 Genomes reference data were excluded from imputation. Imputation was done against all-ethnicity 1000 Genomes haplotypes (excluding monomorphic and singleton sites) using Minimac[36]. For the X chromosome, separate haplotype graphs were built for the

non-pseudoautosomal region and each pseudoautosomal region, and these regions were phased separately. Males and females were imputed together using Minimac2[36], as with the autosomes, treating males as homozygous pseudo-diploids for the non-pseudoautosomal region.

Association test results were computed by linear regression assuming additive allelic effects. For tests imputed dosages rather than best-guess genotypes were used. Covariates for age, gender, the ten five principal components to account for residual population structure were also included into the model. Results for the X chromosome are computed similarly, with male genotypes coded as if they were homozygous diploid for the observed allele.

HLA allele dosages were imputed from SNP genotype data using HIBAG[37]. We imputed alleles for HLA-A, B, C, DPB1, DQA1, DQB1, and DRB1 loci at four-digit resolution. To test associations between HLA allele dosages and phenotypes, we performed logistic or linear regression using the same set of covariates used in the SNP-based GWAS for that phenotype. We performed separate association tests for each imputed allele.

The UK Biobank. The UK Biobank database includes 502,682 participants who were aged from 49–69 years when recruited between 2006 and 2010 from across the UK to take part in the project. The study was approved by the National Research Ethics Committee (REC reference 11/NW/0382). The participants filled out several questionnaires about their lifestyle, environmental risk factors and medical history and gave their informed consent[38]. The participants were invited, through a computerized questionnaire, to answer the question "What best describes your natural hair colour? (If your hair colour is grey, the colour before you went grey)". The participants' answers were used to create a hair color variable with values: 1- blond, 2-red, 3-light brown, 4- dark brown, 5-black and other codes for "other, "Don't know" or "prefer not to answer". The later three values were removed from analyses.

Extracted DNA were then processed in the approximate order received to produce genotype data using the Affymetrix Axiom® platform as described elsewhere (see URLs). Details on genotyping procedure and quality control can be found elsewhere (see URLs). Phasing on the autosomes was carried out using a modified version of the SHAPEIT2[34] program modified to allow for very large sample sizes. The new algorithm uses a divisive clustering algorithm to identify clusters of haplotypes, and then calculates Hamming distances only between pairs of haplotypes within each cluster. Only haplotypes within each cluster are used as candidates for the surrogate family copying states in the HMM model. Imputation was carried out using the same algorithm as is implemented in the IMPUTE2 program. More detailed information on the imputation procedure followed can be found elsewhere (see URLs). Linear models were built for the main GWAS analysis: haircolor ~ genotype + age + sex + PC1:10 + genotyping platform.

Replication cohorts (the VisiGen Consortium). Subjects of European descent participating in any of the 10 studies from the International Visible Trait Genetics Consortium[39] (VisiGen). The VisiGen participants were phenotyped through self-report, and each phenotypic category was assigned a unique numerical value within each cohort. The self-reported hair color categories were however highly heterogeneous across the 10 studies. Therefore, all participants (N=27,865) were genotyped, imputed and analyzed separately by each participating center, using standard techniques described in the **Supplementary Notes**.

STATISTICAL ANALYSES

Meta-analyses. Results from each participating cohort (23andMe and UK Biobank) were standardized to allow for the different scale (six categories of hair color in the former but only five in the latter) and minimize differences arising from the slightly different categorizations of the same phenotype. Both weighted z-score and inverse variance analyses (the latter using standardized linear regression coefficients and standard errors) were calculated, using Metal[40]. The results obtained from both methods were similar and inverse-variance results are reported throughout the manuscript for the discovery cohort. Association effects sizes, standard errors and probabilities were taken from the association analyses software. In cases where the significance of the association exceeded the range of float numbers determined by the system and software, the probabilities were calculated using Mathematica 11.1 computational algebra (Wolfram Research Inc. Champaign, IL). Meta-analyses of the replication cohorts were calculated using the weighted z-score method, to reflect the fact that the phenotypic definitions were not harmonized across the different participating cohorts (please refer to the Supplementary Notes for more detailed population description and phenotypic definitions).

Conditional and explained heritability analyses. The program GCTA[41] was used for the conditional analyses[42] to identify independent effects within associated loci as well as the calculation of the phenotypic variance explained[43] by all polymorphisms, genotyped or imputed, associated with the trait after the conditional analyses. The threshold of significance was set at $5x10^{-08}$ and collinearity threshold was r^2=0.8. These estimates were derived from the UKBB cohort.

Natural selection. Results of three statistical tests for natural selection were obtained from the 1000 Genomes Selection Browser[44]. Results from three selected tests are reported: iHS[45] and two cross-population comparison (XP-EHH tests, CEU vs. YRI and CEU vs CHB) based on extended haplotype homozygosity test [46]. The absolute test scores and the rank scores (-log10 of the centile of the absolute test score across the genome) for each SNP of interest are reported.

Prediction analyses. Using the independently associated SNPs identified in the discovery GWAS meta-analysis (Supplementary Table 9) together with previously reported hair color predicting SNPs from the HIrisPlex System [47,48], we performed hair color prediction modelling in the QIMR, RS, and combined QIMR+RS datasets. For this, we randomly split the QIMR, RS, and combined QIMR+RS data into 80% training sets and 20% validation sets, respectively. This approach assures the use of totally independent datasets for predictive marker discovery, model building, and model validation. Of the 258 independently associated SNPs in the discovery GWAS meta-analysis (see Supplementary Table 9), in 5 SNPs failed imputation quality control in the RS and one in the QIMR and were therefore not included in the models. In the combined QIMR+RS analysis, we used the overlapping set of SNPs. The performance of the prediction models was evaluated in the respective validation datasets using the area under the receiver operating characteristic (ROC) curve (AUC). AUC is the integral of ROC curves which ranges from 0.5 representing total lack of prediction to 1.0 representing perfect prediction. Prediction analyses were conducted in R version 3.2.3 using relevant packages, including , lars, nnet, and pROC.

Effects of variants on gene expression. The potential eQTL effects of the variants of interest was evaluated in all the 57 tissues available at the GTEx[49] portal (URLs). Associations with the levels of expressions of adjacent genes was assessed for all variants identified through the conditional analysis.

Gene set enrichment analyses were carried out on summary results obtained from the meta-analysis of the UK Biobank and 23andMe subjects using the Magenta software[50].

URLs

Descriptionf of the hair color phenotyping in the UK Biobank participants:
https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=1747

Description of the the genotyping procedures for the UK Biobank participants :
http://www.ukbiobank.ac.uk/wp-content/uploads/2014/04/Affymetrix-UKB_WCSGAX-Genotype-Data-Generation.pdf

Genotype imputation and genetic association studies of UK Biobank Interim Data Release, May 2015
http://www.ukbiobank.ac.uk/wp-content/uploads/2014/04/imputation_documentation_May2015.pdf

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