

**Genome-wide association studies identify multiple genetic loci influencing
eyebrow color variation in Europeans**

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TO THE EDITOR,

Eyebrow color shows a high degree of variation in Europeans. Although no heritability estimate has yet been reported, eyebrow color may share a large genetic component with scalp hair color, which has an estimated heritability of up to 90% (Lin et al., 2015). Although phenotypic relationship between eyebrow and scalp hair color clearly exists, such a correlation is not perfect, suggesting the existence of overlapping and unique genetic components for both traits. While previous genome-wide association studies (GWASs) on human eye (Kayser et al., 2008, Liu et al., 2010, Sulem et al., 2008, Sulem et al., 2007), scalp hair (Han et al., 2008, Hysi et al., 2018, Sulem et al., 2007), and skin color (Han et al., 2008, Liu et al., 2015, Sulem et al., 2007, Visconti et al., 2018) have identified multiple DNA variants, no GWAS for eyebrow color has been reported as of yet.

The cohorts related studies included in the current study have been approved by the Medical Ethics Committee of the Erasmus MC, the St. Thomas' Hospital Local Research Ethics Committee, the QIMR Berghofer Human Research Ethics Committee, and the Indiana University Internal Review Board, respectively; and all participants provided written informed consent under protocols reviewed by the corresponding institutions.

The discovery stage meta-analysis of three GWASs for eyebrow color included a total of 6,513 European individuals from three cohorts, the Rotterdam Study (RS, $n=3,114$, mean age 68.48 ± 9.34 , 53.6% female), the TwinsUK study ($n=1,038$, mean

age 59.47±9.50, all females), and the Queensland Institute of Medical Research study (QIMR, n=2,361, mean age 16.43±0.80, 54.0% female, **Table S1**). Eyebrow color was graded into four broad ordinal categories i.e., red, blond, brown, and black using photo-numeric scales (**Table S1**). Detailed phenotype evaluation is provided in **Table S2-S6** and in Supplementary Materials.

The discovery stage meta-analysis of three GWASs identified a total of 355 SNPs at 6 distinct genetic loci showing genome-wide significant association with eyebrow color ($p < 5 \times 10^{-8}$, **Figure 1 & S1, Table S7**). Among these 6 loci, one locus (10q22.2: *C10orf11*) had not been previously associated with any other human pigmentation trait; the top-associated SNP (rs11001536, $\beta = -0.21$, $p = 3.16 \times 10^{-8}$, **Table S7**) is an intronic DNA variant in *C10orf11* (**Figure S2**). The remaining 5 loci have been repeatedly reported with genome-wide significant association with human eye, scalp hair, and/or skin color. These include 15q13.1 (rs7494942, $\beta = 0.22$, $p = 6.36 \times 10^{-58}$ for *HERC2* and rs4778237, $\beta = 0.17$, $p = 7.01 \times 10^{-31}$ for *OCA2*), 16q24.3 (*MC1R* rs75570604, $\beta = -0.25$, $p = 9.88 \times 10^{-52}$), 14q32.12 (*SLC24A4* rs12883151, $\beta = 0.10$, $p = 4.44 \times 10^{-27}$), 20q11.22 (*ASIP* rs6059655, $\beta = -0.11$, $p = 4.60 \times 10^{-10}$) and 5p13.2 (*SLC45A2* rs16891982, $\beta = 0.18$, $p = 2.60 \times 10^{-8}$) (**Table S7**).

The replication was conducted in 2,054 individuals of European origin from an additional United States cohort (US, mean age 25.75±11.39, 68% female, **Table S1**). Five loci highlighted in our discovery GWAS meta-analysis (*SCL45A2*, *SCL24A4*, *HERC2* & *OCA2*, *MC1R*, and *ASIP*) have been successfully replicated in US ($p < 0.05$,

Table S7), and showed consistent allele effects in all four cohorts (**Figure S3**). The significant association for rs11001536 in *C10orf11* highlighted in the discovery GWAS was not replicated in US. This SNP was nominally significant in RS ($\beta=-0.23$, $p=2.16\times 10^{-6}$) and in TwinsUK ($\beta=-0.27$, $p=4.97\times 10^{-3}$), but non-significant in QIMR ($\beta=-0.09$, $p=0.26$) and in US ($\beta=0.07$, $p=0.60$) (**Table S7**). Notably, both cohorts that showed significant association consist of elderly (RS & TwinsUK), while the two datasets not showing significant association were obtained from adolescents (QIMR & US). This suggests that the eyebrow color effect of *C10orf11* may be age-dependent, which warrants further investigation in future studies. The light eyebrow color associated G-allele had a relatively low frequency in Europeans ($f=0.02$) but more frequent in Asians with darker eyebrows (**Figure S4**), potentially explained by different LD structures between these populations. Previous studies suggest that *C10orf11* is an effective melanocyte-differentiation gene, which is known to cause the Oculocutaneous Albinism (OCA) 7 phenotype via a rare nonsense mutation c.580C>T (p.Arg194*, rs587776952) (Gronskov et al., 2013). An additional genome-wide meta-analysis in all four cohorts did not reveal any additional genome-wide significant loci (**Figure S5**).

Among the 138 SNPs from a recently published GWAS meta-analysis on scalp hair color involving almost 300,000 Europeans (Hysi et al., 2018), 7 SNPs showed significant association after the correction for multiple testing (adjusted $p<4.67\times 10^{-4}$), including 15q13.1 *HERC2* rs12913832 ($p=1.12\times 10^{-48}$), 16q24.3 *MC1R* rs1805007

($p=1.25\times 10^{-47}$), 14q32.12 *SLC24A4* rs17184180 ($p=1.67\times 10^{-26}$), 20q11.22 *ASIP* rs6059655 ($p=4.60\times 10^{-10}$), 5p13.2 *SLC45A2* rs16891982 ($p=2.60\times 10^{-8}$), 6p25.3 *IRF4* rs12203592 ($p=3.47\times 10^{-6}$), and 1q32.1 *DSTYK* rs2369633 ($p=5.03\times 10^{-5}$) (**Table S8**).

The first 6 SNPs all have known effect on human pigmentation traits. The last SNP was only recently identified in association with hair color (Hysi et al., 2018).

In a subset of RS ($n=1,656$), we compared the 8 top-associated SNPs at the 7 genetic loci that were highlighted with significant eyebrow color association in our GWAS and candidate gene study (**Table S9**). In general, the contributions of the 8 SNPs to scalp hair color variation were slightly larger than their impact on eyebrow color variation (**Figure S6**). *MC1R* rs1805007 showed much larger contribution to scalp hair color than eyebrow color, likely explained by an aging effect on red eyebrow color since the scalp hair color information in RS was ascertained from questionnaire on “hair color when young”. *SLC45A2* rs16891982 was the only DNA variant of the 8 tested that showed slightly larger contribution to eyebrow color than to scalp hair color. These results suggest that the prediction accuracy for eyebrow color should be at a similar level to that for hair color in the same sample set under equal phenotype accuracy.

An eyebrow color prediction model was trained in 3,114 RS subjects and validated in 779 independent RS subjects not included in the GWAS. Red eyebrow was excluded from the prediction analysis since none of these individuals had the phenotype. A model including 25 SNPs achieved prediction accuracies expressed as

AUC at 0.701 (95% CI: 0.621-0.781) for blond, 0.620 (95% CI: 0.576-0.658) for brown, and 0.674 (95% CI: 0.633-0.709) for black eyebrows (**Figure 2a & 2b & S7, Table S10 & S11**). The AUC values reported here for eyebrow color are lower compared to those previously reported for scalp hair color with the 22-SNP HIRISplex model, which ranged between 0.75 and 0.92 for the four scalp hair color categories used (Walsh et al., 2014). This discrepancy can be explained by our dataset lacking four important rare *MC1R* SNPs (which are used in the HIRISplex model), and an aging effect that decreases phenotype quality, particularly for red color. With the mid-ranged accuracy level, our 25-SNP model provided highly confident prediction results for ~7% of the validation set, e.g. those with high (>0.80) or low (<0.20) prediction probabilities of certain eyebrow color type (**Figure 2c**). Applying this model to 2,504 subjects from the 1000 Genomes Project revealed that prediction outcomes were generally consistent with knowledge about the global distribution of eyebrow color variation (**Figure 2d & S8**). More detailed prediction results are provided in the **Supplementary Materials**.

In conclusion, this first eyebrow color GWAS in Europeans highlighted 6 genome-wide significant genetic loci harboring 6 well-known pigmentation genes including *ASIP*, *HERC2*, *MC1R*, *OCA2*, *SLC24A4*, *SLC45A2*, and a gene to our knowledge previously unreported, *C10orf11*. The finding at *C10orf11* warrants further investigations in European subjects with different age distributions. A candidate gene study suggested the involvement of two additional known pigmentation genes *DSTYK*

and *IRF4* in human eyebrow color. This DNA-based eyebrow color prediction model is useful in future forensic applications.

Conflict of interests

The authors declare that they have no competing interests.

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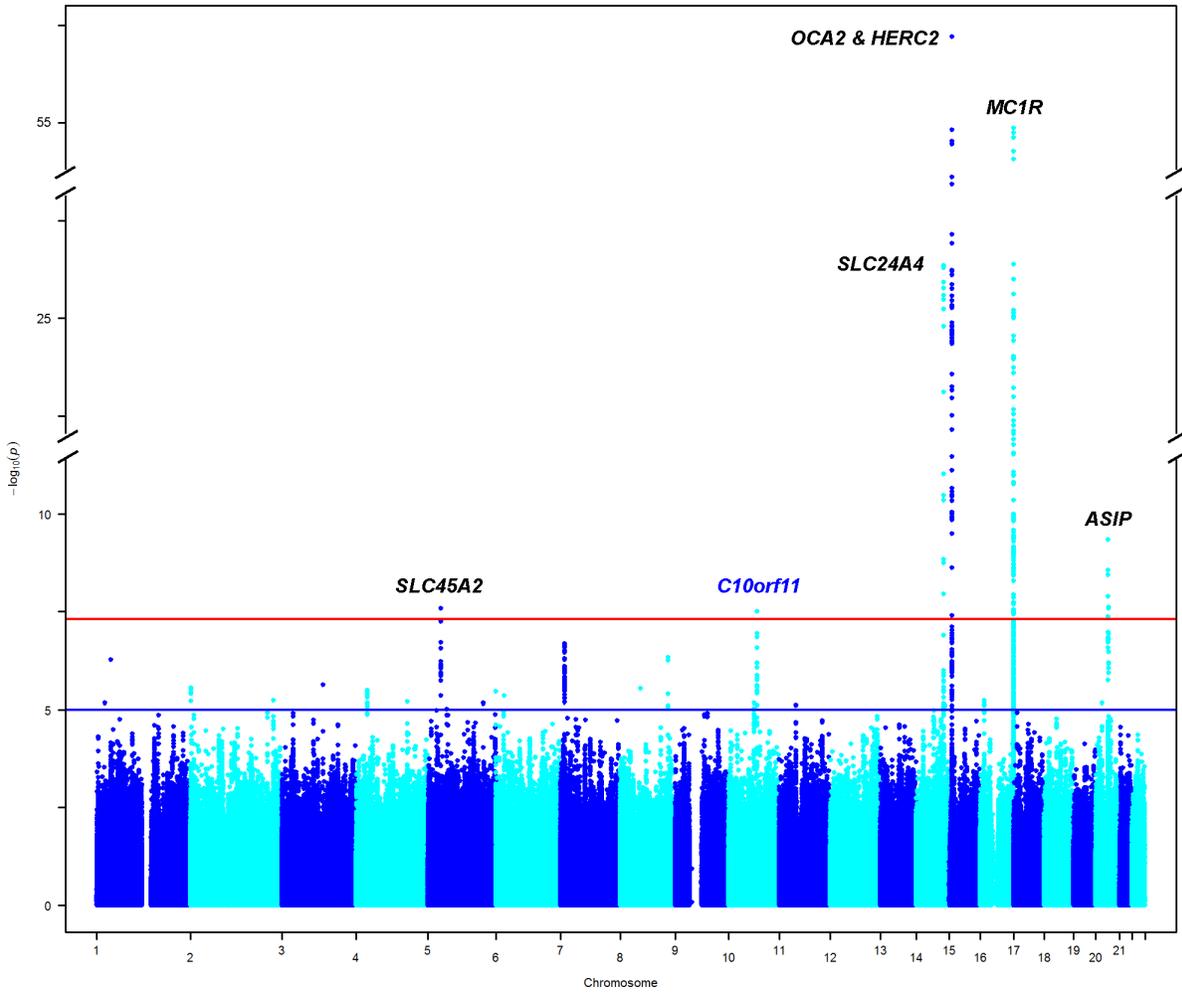
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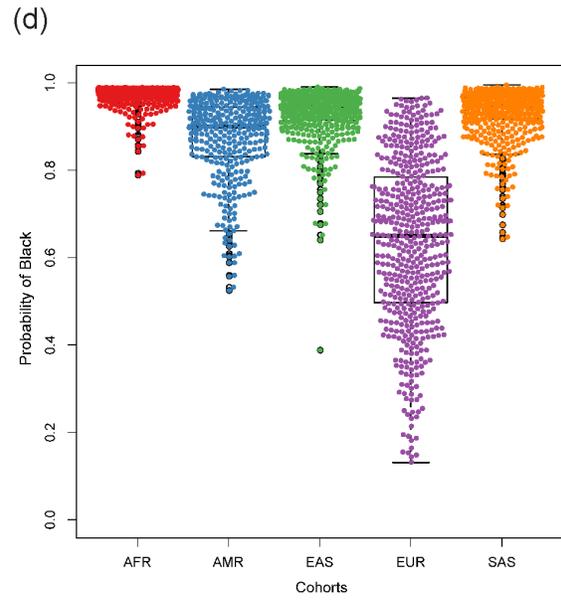
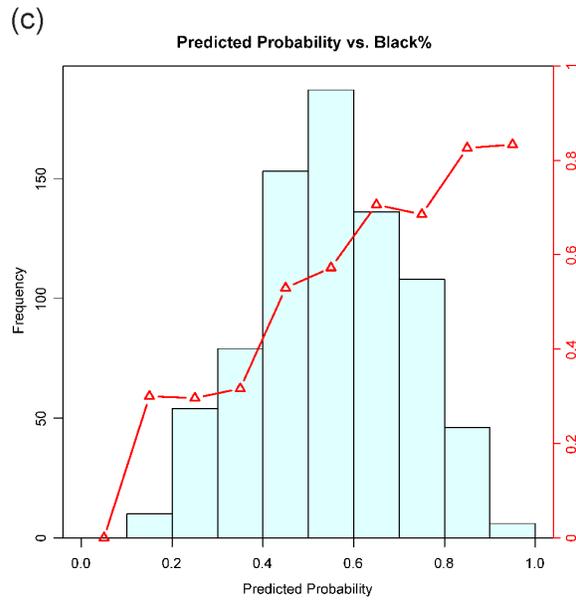
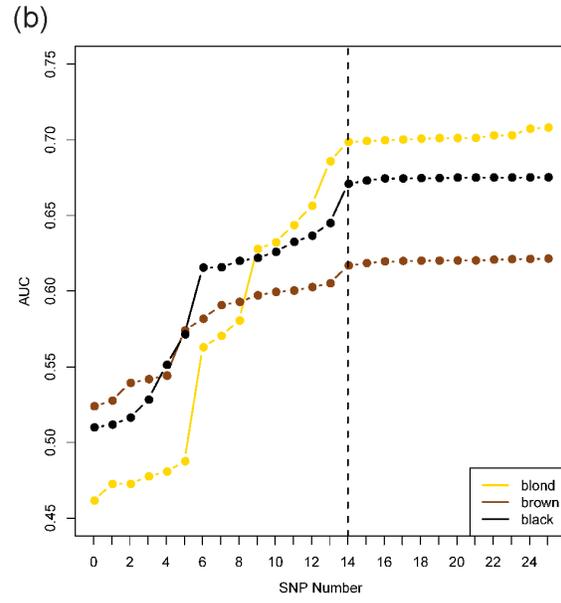
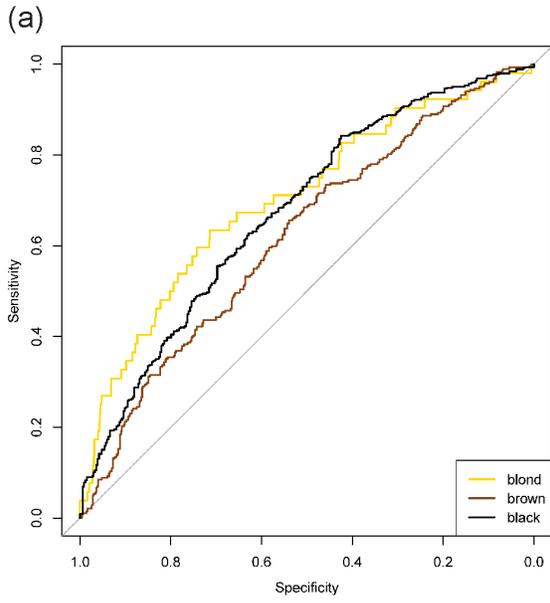
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Figure 1. Manhattan plot of the discovery stage meta-analysis results for human eyebrow color from three European GWASs (RS, TwinsUK and QIMR, total N=6,513). The $-\log_{10} P$ values for association were plotted for each SNP according to chromosomal positions (genome assembly GRCh37.p13). Previously known pigmentation genes were marked using black text and gene to our knowledge previously unreported were highlighted in blue. The red and blue lines, respectively, correspond to the thresholds for genome-wide significance ($p=5\times 10^{-8}$) and suggestive significance ($p=1\times 10^{-5}$).

Figure 2. Genetic predictions of eyebrow color. **(a)** Illustration of the prediction performance of the set of 25 SNPs for eyebrow color prediction model using ROC curves with AUC estimates in independent individuals (N=779). **(b)** AUC was plotted against the number of SNPs included in the multinomial logistic model. The top 14-SNP annotation and prediction ranks are provided in **Table S11**. **(c)** Frequency (left y-axis) of predicted probability (x-axis) for black eyebrow and percentage (right y-axis) of non-black eyebrow in all 779 RS samples. **(d)** The distribution of predicted black eyebrow color probabilities for 2,504 subjects from the 1000-Genomes Project panel; samples are grouped according to the 5 continents they originate from: AFR-Africans, AMR-Native Americans, EAS-East Asians, EUR-Europeans, and SAS-South Asians.



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