Zhou et al., 2013), however, the specificity of the *abcc6a* zebrafish model was not validated by an independent gene-editing tool.

In their recent paper, Van Gils et al. (2018), based on work by others in the field (Bedell et al., 2011; Kok et al., 2015), demonstrated that the nonspecific phenotype observed in our study was most likely a consequence of high dosage of *abcc6a* morpholino, and this can be counteracted by co-injection of p53 morpholino. Van Gils et al. (2018) also generated a complete *abcc6a* knockout zebrafish model using CRISPR/Cas9 with ectopic mineralization phenotype (Van Gils et al., 2018). We are pleased that the progress in the field has clarified some aspects of our early pioneering work, which attempted to develop the first zebrafish model for PXE, well before techniques such as CRISPR/Cas9 were available.

As indicated in our recent Commentary on this topic (Li and Uitto, 2018), zebrafish models offer a facile and cost-efficient platform for high throughput screen of potential pharmacologic agents to counteract the clinical phenotypes of heritable connective tissue and skin disorders, such as ectopic mineralization in PXE using *abcc6a* mutant zebrafish. In this regard, we would like to reiterate the call by Vanchieri (2001) to "Move Over, Mice: Make Way for the Woodchucks, Ferrets, and Zebrafish." However, as noted in our Commentary (Li and Uitto, 2018), the zebrafish mineralization models characterized by Van Gils et al. (2018) do not fully recapitulate the phenotypes of PXE patients; these models show little or no evidence of skin, eye, or vascular mineralization, the clinico-pathological hallmarks of PXE, and instead, the changes mostly involve skeletal structures. Therefore, we suggest that after initial high throughput screen of potential anti-mineralization compounds in zebrafish, their efficacy should be tested in rodent models of PXE (knockout mice and rats) that we have developed to accurately and reproducibly recapitulate the features of PXE (Klement et al., 2005; Li et al., 2017).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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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 a 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. Although previous genome-wide association studies (GWASs) on human eye (Kayser et al., 2008; Liu et al., 2010; Sulem et al., 2007, 2008), 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.,

Abbreviations: AUC, area under the curve; CI, confidence interval; GWAS, genome-wide association study; QIMR, Queensland Institute of Medical Research; RS, Rotterdam Study; SNP, single nucleotide polymorphism

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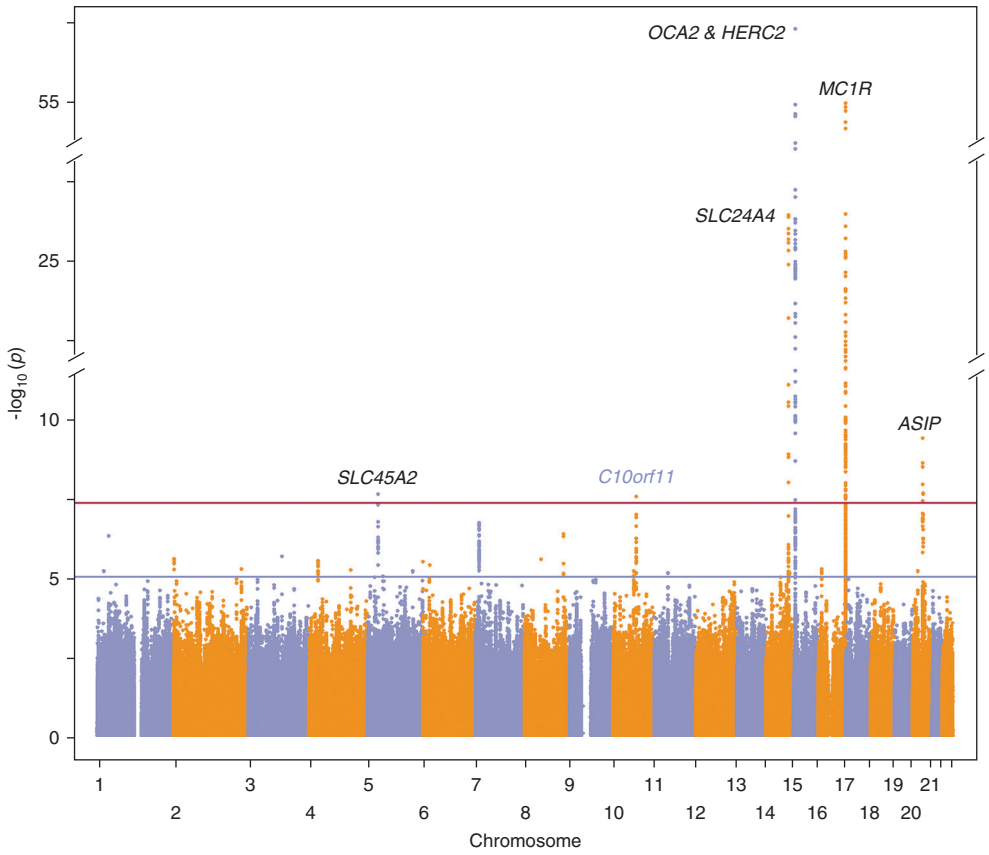

Figure 1. Manhattan plot of the discovery stage meta-analysis results for human eyebrow color from three European GWASs (RS, TwinsUK, and QIMR (N = 6,513). The −log10 *P*-values for association were plotted for each SNP according to chromosomal positions (genome assembly GRCh37.p13). Previously known pigmentation genes are marked with black text, and gene to our knowledge previously unreported is highlighted in violet. The red and violet lines, respectively, correspond to the thresholds for genome-wide significance ($P = 5 \times 10^{-8}$) and suggestive significance ($P = 1 \times 10^{-5}$). GWAS, genome-wide association study; QIMR, Queensland Institute of Medical Research; RS, Rotterdam Study; SNP, single nucleotide polymorphism.

2018) have identified multiple DNA variants, no GWAS for eyebrow color has been reported as of yet.

The cohort-related studies included in this study were approved by the medical ethics committee of the Erasmus University Medical Center, the St. Thomas' Hospital local research ethics committee, the Queensland Institute of Medical Research (QIMR) Berghofer human research ethics committee, and the Indiana University internal review board. 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 years, 53.6% female), the TwinsUK study (n = 1,038, mean age = 59.47 ± 9.50 years, 100% female), and the QIMR study (n = 2,361, mean age = 16.43 ± 0.80 years, 54.0% female) (see Supplementary Table S1 online). Eyebrow color was graded into four broad ordinal categories (red, blond, brown, and black) by using photonumeric scales (see Supplementary Table S1). Detailed phenotype evaluation is provided in Supplementary Tables S2–S6 and the Supplementary Materials online.

The discovery stage meta-analysis of three GWASs identified a total of 355 single nucleotide polymorphisms (SNPs) at six distinct genetic loci showing genome-wide significant association with eyebrow color ($P < 5 \times 10^{-8}$) (Figure 1 and see Supplementary Figure S1 and Supplementary Table S7 online). Among these six loci, one locus (10q22.2: *C10orf11*) had not been previously associated with any other human pigmentation trait; the top-associated SNP (rs11001536; $\beta = -0.21$, $P = 3.16 \times 10^{-8}$) (see Supplementary Table S7) is an intronic DNA variant in *C10orf11* (see Supplementary Figure S2 online). The remaining five loci have been repeatedly reported to have 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, $\beta = 0.18$, $Ps = 2.60 \times 10^{-8}$) (see Supplementary Table S7).

The replication was conducted in 2,054 individuals of European origin from an additional US cohort (mean age = 25.75 ± 11.39 years, 68% female) (see Supplementary Table S1). Five loci highlighted in our discovery GWAS meta-analysis (*SCL45A2*,

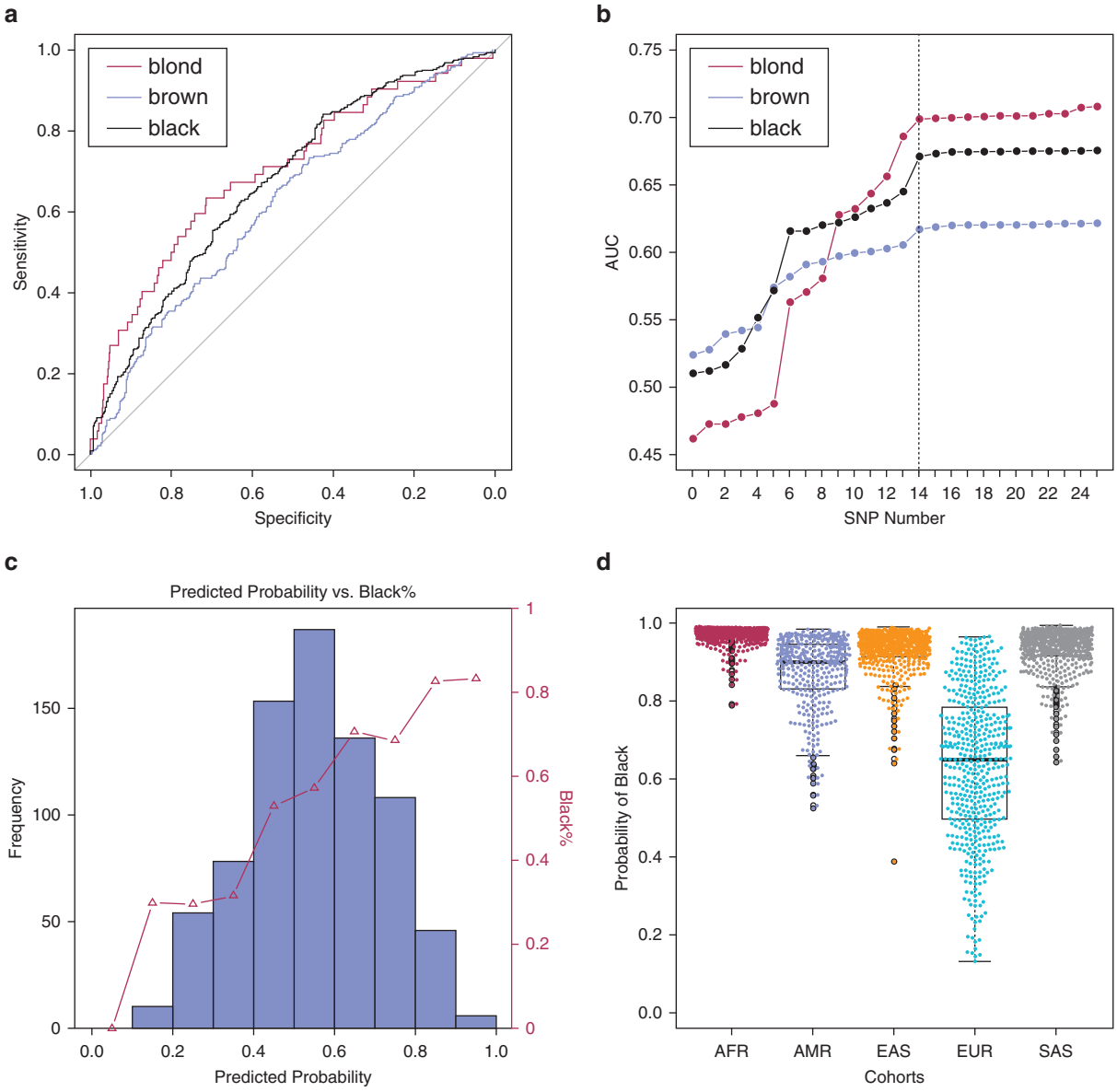

Figure 2. Genetic predictions of eyebrow color. **(a)** The prediction performance of the set of 25 SNPs for eyebrow color prediction model using ROC curves with AUC estimates in independent individuals (N = 779). **(b)** AUC was plotted against the number of SNPs included in the multinomial logistic model. The top 14 SNP annotation and prediction ranks are provided in Supplementary Table S11. **(c)** Frequency (left *y*-axis) of predicted probability (*x*-axis) for black eyebrow and percentage (right *y*-axis) of nonblack eyebrow in all 779 RS samples. **(d)** The distribution of predicted black eyebrow color probabilities for 2,504 participants from the 1000 Genomes Project panel; samples are grouped according to place of origin. AFR, Africans; AMR, Native Americans; AUC, area under the curve; EAS, East Asians; EUR, Europeans; ROC, receiver operating characteristic; RS, Rotterdam Study; SAS, South Asians; SNP, single nucleotide polymorphism.

SCL24A4, *HERC2* and *OCA2*, *MC1R*, and *ASIP*) have been successfully replicated in the US cohort ($P < 0.05$) (see Supplementary Table S7) and showed consistent allele effects in all four cohorts (see Supplementary Figure S3 online). The significant association for rs11001536 in *C10orf11* highlighted in the discovery GWAS was not replicated in the US cohort. This SNP was nominally significant in the RS ($\beta = -0.23$, $P = 2.16 \times 10^{-6}$) and TwinsUK ($\beta = -0.27$, $P = 4.97 \times 10^{-3}$) cohorts but was nonsignificant in the QIMR ($\beta = -0.09$, $P = 0.26$) and the US ($\beta = 0.07$, $P = 0.60$) cohorts (see Supplementary Table S7).

Notably, both cohorts (RS and TwinsUK) that showed significant association consist of older individuals, whereas the two datasets not showing significant association (QIMR and US) consist of adolescents. 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 was more frequent in Asians with darker eyebrows (see Supplementary Figure S4 online), potentially explained by different linkage disequilibrium structures between these populations. Previous studies suggest that *C10orf11* is an effective melanocyte differentiation gene, which is known to cause the oculocutaneous albinism (i.e., 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 show any additional genome-wide significant loci (see Supplementary Figure S5 online).

Among the 138 SNPs from a recently published GWAS meta-analysis on scalp hair color involving almost 300,000 Europeans (Hysi et al., 2018), seven 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 ($P = 5.03 \times 10^{-5}$) (see Supplementary Table S8 online). The first six SNPs all have known effects on human pigmentation traits. The last SNP was only recently identified in association with hair color (Hysi et al., 2018).

In a subset of the RS cohort (n = 1,656), we compared the eight top associated SNPs at the seven genetic loci that were highlighted with significant eyebrow color association in our GWAS and candidate gene study (see Supplementary Table S9 online). In general, the contributions of the eight SNPs to scalp hair color variation were slightly larger than their impact on eyebrow color variation (see Supplementary Figure S6 online). *MC1R* rs1805007 showed a much larger contribution to scalp hair color than eyebrow color, likely explained by an aging effect on red eyebrow color, because the scalp hair color information in the RS cohort was ascertained from a questionnaire item on "hair color when young." *SLC45A2* rs16891982 was the only DNA variant of the eight tested that showed a 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 participants and validated in 779 independent RS participants not included in the GWAS. Red eyebrow color was excluded from the prediction analysis because none of these individuals had the phenotype. A model including 25 SNPs achieved prediction accuracies expressed as area under the curve (AUC) of 0.701 (95% confidence interval [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 and b, and see Supplementary Figure S7 and Supplementary Tables S10 and S11 online). The AUC values reported here for eyebrow color are lower than 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 data set 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 midrange accuracy level, our 25-SNP model provided highly confident prediction results for approximately 7% of the validation set, for example, those with high (>0.80) or low (<0.20) prediction probabilities of certain eyebrow color type (Figure 2c). Applying this model to 2,504 participants from the 1000 Genomes Project showed that prediction outcomes were generally consistent with knowledge about the global distribution of eyebrow color variation (Figure 2d, and see Supplementary Figure S8 online). More detailed prediction results are provided in the Supplementary Materials.

In conclusion, this eyebrow color GWAS in Europeans (the first, to our knowledge) highlighted six genome-wide significant genetic loci harboring six well-known pigmentation genes (*ASIP*, *HERC2*, *MC1R*, *OCA2*, *SLC24A4*, *SLC45A2*) and a gene to our knowledge previously unreported (*C10orf11*). The finding at *C10orf11* warrants further investigations in European individuals 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 INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.12.029.

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Susceptibility Loci for Tanning Ability in the Japanese Population Identified by a Genome-Wide Association Study from the Tohoku Medical Megabank Project Cohort Study

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TO THE EDITOR

The ability of human pigmentation varies within and across populations. The geographic variation in human skin pigmentation is hypothesized to be a result of adaptation to the amount of UV exposure, and the high heritability of pigmentation on various traits such as hair and eye color supports the hypothesis. (Frisancho et al., 1981; Harrison and Owen, 1964; Jablonski and Chaplin, 2000). Genome-wide association studies (GWAS) for skin pigmentation or skin tanning showed several SNPs that affect skin pigmentation depending on the studied population and traits (see Supplementary Table S1 online). The Fitzpatrick phototyping scale is widely used to assess human skin color and pigmentation by classifying them into six skin types (Fitzpatrick, 1988). Japanese are mostly categorized as type III or IV in the Fitzpatrick phototyping scale. Satoh and Kawada's Japanese skin types (JST) classification classify Japanese skin into three types based on the susceptibility to sunburn and the ability to tan: J-I, burns easily and tans minimally; J-II, burns moderately and tans moderately; and J-III, burns slightly and tans markedly (Satoh and Kawada, 1986). The correlation between the self-reported questionnaire for the JST classification and minimal erythema dose has been validated, and the JST classification is widely used in skin type tests for Japanese studies. We performed a GWAS for JST using data sets from the prospective cohort study of the Tohoku Medical Megabank Project (TMM). The GWAS included 9,187 Japanese individuals: 4,475 individuals in Miyagi prefecture and 4,712 individuals in Iwate

Abbreviations: GWAS, genome-wide association study; JST, Japanese Skin Types; PCA, principal component analysis; SNP, single nucleotide polymorphism; TMM, Tohoku Medical Megabank Project

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