Linkage and Association Analysis of Spectrophotometrically Quantified Hair Color in Australian Adolescents: the Effect of *OCA2* and *HERC2*

Sri N. Shekar[1,4], David L. Duffy[1], Tony Frudakis[2], Richard A. Sturm[3], Zhen Z. Zhao[1], Grant W. Montgomery[1] and Nicholas G. Martin[1]

Genetic studies of pigmentation have benefited from spectrophotometric measures of light–dark hair color. Here we use one of those measures, absorbance at 650 nm, to look for chromosomal regions that harbor genes affecting hair pigmentation. At 7p15.1, marker D7S1808 was suggestive of linkage to light–dark hair color (LOD ≈ 2.99). Marker D1S235 at 1q42.3 was suggestive of linkage to hair color (light–dark or blonde-black continuum) (LOD ≈ 2.14). However, the most consistent linkage peak was over the gene oculocutaneous albinism type II (*OCA2*) on chromosome 15. Linkage analysis of both spectrophotometrically quantified and ordered ratings of hair color had LOD scores about 1.2, significant because of the almost perfect concordance. A quantitative transmission disequilibrium test between light–dark hair color and 58 single nucleotide polymorphisms in *OCA2* showed that the SNPs rs4778138 (also called rs11855019) and rs1375164 were associated with significantly darker hair color ($P \approx 3 \times 10^{-4}$ and $P \approx 0.03$ after correction for multiple testing, respectively). These two SNPs explain 1.54 and 0.85% of variation in the A650t index, respectively.

Journal of Investigative Dermatology (2008) **128,** 2807–2814; doi:10.1038/jid.2008.147; published online 5 June 2008

INTRODUCTION

When studying variation in a trait, particularly variation due to genetic influences, it is important to have an accurate measure of the trait. Investigations into hair color have progressed from subjective visual ratings to spectrophotometric measures and biochemical quantifications. The first studies categorized hair color by visual inspection (Davenport and Davenport, 1909), an approach standardized in the Fischer–Saller Haarfarbentafel scale using 30 swatches (Hanna, 1956). Interested in using spectrophotometry, Harrison and Owen (1964) showed that the wavelength with the greatest power to distinguish between different quantities of dermal melanin is approximately 650 nm. Wavelengths about 650 nm are also used in the melanin index to quantify dermal melanin (Shriver and Parra, 2000).

With the development of chemical methods (Wakamatsu and Ito, 2002), studies investigated the relationship between biochemically quantified eumelanin and spectrophotometric reflectance of human hair. Ozeki *et al.* (1995) show that total melanin (eu and pheomelanin) is best quantified at 500 nm and Ozeki *et al.* (1996) showed that the ratio of eumelanin/total melanin is best represented by the ratio of 650 nm:500 nm. There are a number of methods to quantify eumelanin with great accuracy such as electron paramagnetic resonance (reviewed by Ito *et al.*, 2000). However, the resources required make them infeasible for large epidemiologic studies. So, many large studies of melanin use the melanin index (for example, Shriver *et al.*, 2003 and Parra *et al.*, 2004).

We expect the majority of genetic influences explaining variation in eumelanin to be different from those influences explaining pheomelanin. In a previous study (Shekar *et al.*, 2008), we tested whether it was possible to partition variation in hair color due to eumelanin from that due to pheomelanin. This sample (Shekar *et al.*, 2008), contained 3,180 individuals with ordered ratings of hair color. Of those, 1,730 had spectrophotometric curves derived from hair samples. The best measure to explain the ordered categories, namely fair/blonde, light brown, dark brown, and black was reflectance

[1]*Genetic Epidemiology Unit, Queensland Institute of Medical Research, Brisbane, Queensland, Australia;* [2]*DNAPrint, Genomics Inc, Sarasota, Florida, USA;* [3]*Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia and* [4]*School of Medicine, University of Queensland, Brisbane, Queensland, Australia*

Investigations undertaken in Brisbane, Queensland, Australia

Correspondence: Dr David L. Duffy, Genetic Epidemiology Unit, Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Brisbane, Queensland 4029, Australia. E-mail: david.duffy@qimr.edu.au

Abbreviations: A650t, reflectance at 650 nm, transformed for normality; HERC2, HECT Domain and RCC1-Like Domain 2; OCA2, oculocutaneous albinism type II gene; QTDT, quantitative transmission disequilibrium test; QTL, quantitative trait locus; SNP, single nucleotide polymorphism

Received 17 January 2008; revised 11 March 2008; accepted 12 March 2008; published online 5 June 2008

at 650 nm, transformed for normality (A650t). By removing individuals classified as having red hair, we obtained an estimator of eumelanin quantity. This A650t measure correlated −0.99 with the formula for the melanin index applied to reflectance of the hair samples. However, compared with the melanin index, the A650t index only uses one wavelength, a benefit when using some spectrophotometric devices.

A classical twin analysis of ordered the ratings of hair color showed that there were additive genetic and common environmental influences causing variation in hair color. The complementary analysis of the A650t index showed that some of those additive genetic influences were qualitatively different between males and females (Shekar *et al.*, 2008). An early linkage study showed that brown hair was linked to 15q (Eiberg and Mohr, 1996). Recently, it has been found that the oculocutaneous albinism type II gene (*OCA2*) in this region is significantly associated with eye color (Rebbeck *et al.*, 2002; Frudakis *et al.*, 2003, 2007; Zhu *et al.*, 2004; Jannot *et al.*, 2005; Posthuma *et al.*, 2006; Duffy *et al.*, 2007). One 3-SNP haplotype (rs7495174, rs6497268 (now rs4778241), and rs11855019 (now rs4778138)) in the first intron of *OCA2* explains 74% of variation in blue: non-blue eye color (Duffy *et al.*, 2007). The same authors have shown that a single SNP rs12913832, in the 86th intron of the 5′ neighboring gene *HERC2* (HECT Domain and RCC1-Like Domain 2), almost completely accounts for blue/brown eye color (Sturm *et al.*, 2008), a finding replicated by Eiberg *et al.* (2008). The fact that this SNP is predicted to create/destroy a helicase-like transcription factor-binding site adds weight to the evidence that this is the causal SNP, although further functional studies are needed.

In the present paper, we look for specific genes to explain the genetic variation in light–dark hair color. We also investigate what effect genetic variations in the *OCA2–HERC2* region have on variation in spectrophotometrically approximated eumelanin quantity in human hair.

RESULTS

Linkage analysis

The logarithm of odds (LOD) scores for each of the 761 autosomal markers for the two linkage analyses of the A650t index and ordered hair color are shown in Figure 1. Markers that produced LOD scores greater than 1.8 are listed in Table 1. The most notable location with an overlap in linkage peaks between the two analyses is near *OCA2* on chromosome 15. Although the LOD score for this peak is relatively small (LOD ≈ 1.2), there is almost perfect concordance between the analyses. There also appears to be some concordance on chromosome 11 around the Tyrosinase gene (*TYR*). The peak with the greatest LOD score in the linkage analysis of the A650t index was at marker D7S1808 on 7p15.1. The LOD score of 2.99 is suggestive of linkage to this region but less than the value indicating significant linkage. However, the fact that there is no linkage signal for the ordered rating at this location may indicate that this is a chance finding. On the X chromosome, the highest LOD score was 0.24 for the A650t index at marker CXS318.

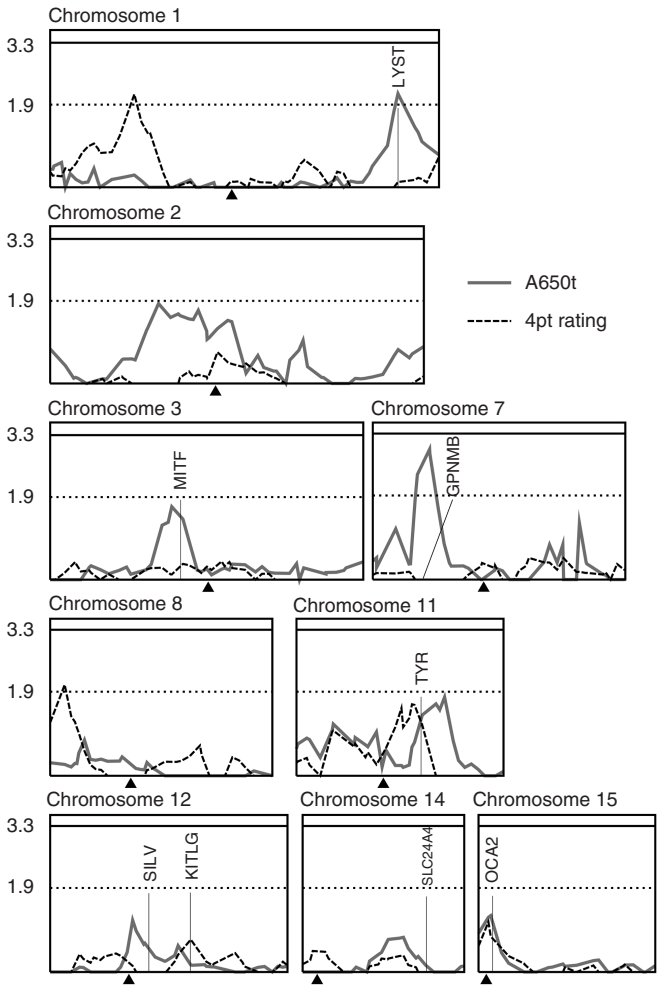

Figure 1. Linkage analysis of hair reflectance at 650 nm (A650t index) and analysis of ordered, rated, hair color. The *x* axis is chromosomal position (▲ marks centromere). The *Y* axis is LOD score with both the suggestive threshold score, 1.9, and the significant threshold score, 3.3, indicated. Results for all 22 autosomes can be found in Figure S1.

Table 1. Cytogenetic locations with LOD scores above 1.8 for linkage analyses of the A650t index and ordered color

Cytogenetic location	Marker	A650t	Ordered color
1p34.3	D1S255	0.28	2.13
1q42.3	D1S235	2.14	0.12
2p16.3	D2S1352	1.83	0.00
7p21.1	D7S2210	1.89	0.00
7p15.3	D7S493	2.41	0.00
7p15.1	D7S1808	2.99	0.00
	D7S516	2.89	0.00
	D7S817	1.66	0.00
8p23.1	D8S277	0.26	2.08
11q23.1	D11S1391	1.80	0.00

Adjacent markers within the same linkage peak are indicated.

Association analysis with *OCA2* and *HERC2*

Association analyses were performed between *OCA2* and hair color. Considering the difference in allele frequencies for *OCA2* SNPs between populations (Lee *et al.*, 1995; Shriver *et al.*, 2003), it is expected that population stratification may confound any association analysis of *OCA2* and hair color. Although the individuals in this sample indicated that their ancestors (great grandparents) were predominantly from the British Isles and Europe, there were a handful of families that had at least one great grand parent with Aboriginal or Papua New Guinean ancestry. These individuals were removed from the association analysis. To further determine whether there may be heterogeneity in population structure between the remaining families, tests of total association and population stratification with A650t were performed on the alleles of the 761 markers in the genome scan (Devlin *et al.*, 2001) using the quantitative transmission disequilibrium test (QTDT). Approximately 5.4% of these tests, in both analyses, were significant at the 0.05 level. This number is no more than would be expected by chance, providing some evidence that there is no significant population stratification within this sample.

Of the 58 *OCA2* SNPs informative for association analysis, tests of total association were performed in QTDT (Abecasis *et al.*, 2000) with red hair color as a covariate (1 if the individual was categorized as having red hair and 0 otherwise) and A, C, and E as sources of variation in addition to a quantitative trait locus (QTL) effect. The results are presented in Figure 2 (Barrett *et al.*, 2005). After correcting for the multiple tests performed, the C allele of the SNP rs4778138 (also called rs11855019) was significantly associated with a mean difference in the A650t index ($P \approx 3 \times 10-4$ after Bonferroni correction). This C allele, with a frequency of 0.13 in this sample, is associated with darker hair (Figure 3) and brown eye color (Duffy *et al.*, 2007). *Post hoc* analysis showed that the frequency of this allele in the 13 individuals, for whom greater than half their great grandparents were Papua New Guinean, was 0.86. This is consistent with a major role in pigmentation. Using the frequency of this allele in the founders and the effect of the variant on the mean, it was calculated that this SNP explains 1.54% of variation in the A650t index in our sample (after the removal of those without European ancestry). The mean heterozygote value appeared to deviate from that expected in a purely additive model toward the mean value for those that were homozygous for the C allele. The dominance option in QTDT performs a test of association that includes an additional parameter allowing the heterozygote mean to deviate from a point midway between the two homozygote means. That is, this allows for one allele to be dominant or semidominant. This model was also significantly associated with the A650t index ($P \approx 1.3 \times 10-3$ after Bonferroni correction).

The SNP rs1375164 was also significantly associated with a mean difference in the A650t index after correcting for multiple testing ($P \approx 0.03$). The A allele, with a prevalence of 0.22 in this sample, was associated with darker hair. In the 13 individuals with Papua New Guinean ancestry, *post hoc* analysis showed that the frequency of the A allele was 0.82. The proportion of variation in the A650t index explained by this allele is 0.85%.

A test was performed to determine whether the 3-SNP haplotype (rs7495174, rs6497268, and rs11855019) found by Duffy *et al.* (2007) to have an effect on blue eye color compared with non-blue eye color also influences light–dark hair color. A model was fitted using the computer program Mx (v1.54a) to compare the mean difference in A650t index values between those with the TGT haplotype compared with other genotypes. Those with the TGT haplotype had significantly darker hair color (Figure 3). To compare this result against the use of an ordered rating, we show the deviation in threshold values (*z*-scores) for ordinal modeling of hair color categories (Figure 3). Visual inspection shows a similar effect size.

Association analyses were performed in QTDT on the rs1129038 and rs12913832 SNPs in *HERC2* using the same model for the *OCA2* SNPs above. Both SNPs, which are in high linkage disequilibrium (Figure 2), had a *P*-value of $3 \times 10-3$ (before correction for multiple testing) for a test of association with A650t. The proportion of variation in the A650t index explained by rs1129038 and rs12913832 is 0.76 and 0.74%, respectively.

DISCUSSION

Here, we use linkage analyses to search for genomic locations of major influence on interindividual variation in light–dark hair color. The highest peak for the linkage analysis of the A650t index is at 7p15.1, where marker D7S1808 had a LOD score of 2.99. Some 4.7 Mb from this marker is the Glycoprotein NMB gene (*GPNMB*). The encoded transmembrane protein is 33% similar to the protein encoded by Silver (*SILV*), a gene important to melanosome biogenesis (reviewed by Theos *et al.*, 2005). A mutation in the mouse *GPNMB* gene causes iris pigment dispersion, causing pigment granules from the iris to be deposited on the tubercular meshwork of the eye (Anderson *et al.*, 2002). It was suggested that the mutations in *GPNMB* alter melanosomes, allowing intermediates of melanin production to escape. The lack of a signal at this locus for ordered ratings does not increase our confidence in this finding, although it may indicate the superior measurement properties of spectrophotometric measures over subjective ratings. Only replication studies will resolve this issue.

Marker D1S235 at location 1q42.3 had a LOD score of 2.14. This marker is within the lysosomal trafficking regulator gene (*LYST*). Mutations in this gene lead to decreased hair pigmentation, classified as Chediak–Higashi syndrome (Barrat *et al.*, 1996). This is homologous to the beige mutation in mice (Jackson, 1997). The linkage peak at 3p13 (D3S1566, LOD = 1.65) is approximately 0.25 Mb from the microphthalmia-associated transcription factor gene (*MITF*), a gene essential for melanocyte differentiation in mice (Levy *et al.*, 2006). A genomewide association of pigmentation in an Icelandic population found SNPs in five genes associated with hair color classified as either blonde or brown (Solute carrier family 24, member 4 (*SLC24A4*), Kit Ligand (*KITLG*), *TYR*, *OCA2*, and *MC1R*; Sulem *et al.*, 2007). In our analysis,

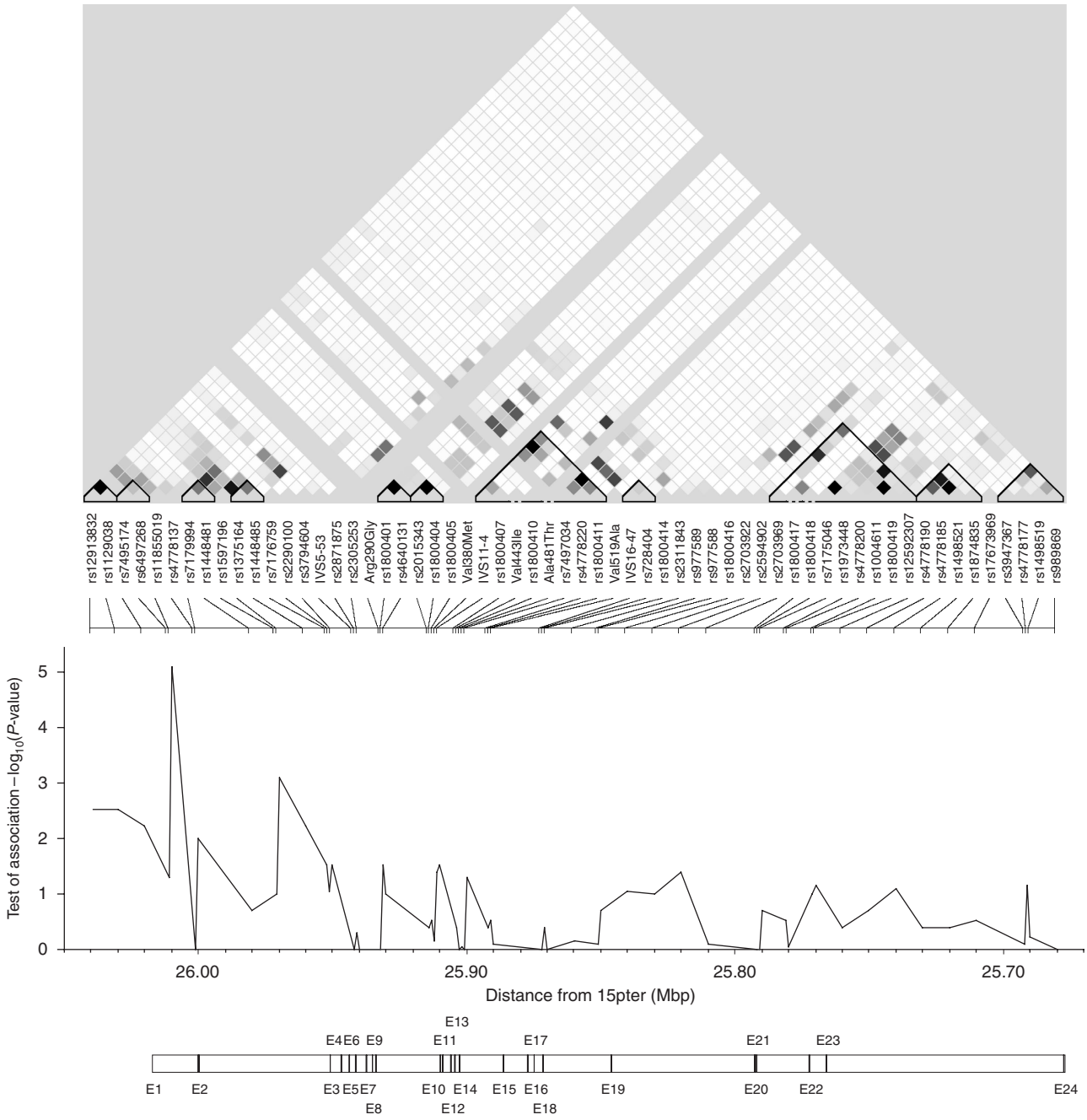

Figure 2. Association analysis. Significance levels for tests of total association between SNPs in *OCA2* and *HERC2* and the A650t index. Upper panel shows the r^2 between typed markers. An exon map for *OCA2* is shown below. SNPs close together have been separated by 0.001 Mb for clarity.

peaks near the *TYR* showed LOD scores of approximately 1.6 for both the A650t index and ordered hair color. As expected, with the removal of individuals with red hair from our sample, markers near the *MC1R* at the q terminal end of chromosome 16 give only a small signal (LOD $\approx$ 0.21).

Linkage signals for spectrophotometrically quantified and ordered ratings of hair color were congruent at marker D15S1002. Although the LOD score was not large, it is 0.15 Mb from *OCA2*. Association analysis showed the rs4778138 and rs1375164 SNPs of *OCA2* contributed 1.54 and 0.85%, respectively, of variation in the A650t index. The average A650t index value for those heterozygous for the rs4778138 deviated from that expected in a purely additive inheritance model, confirming semidominance for this SNP. Our analysis to determine the sources of these genetic effects found that the TGT haplotype of the rs7495174, rs6497268, and rs11855019/rs4778138 SNPs were significantly associated with lighter hair, similar to their association with eye color in this sample (Duffy *et al.*, 2007). However, the effect of *OCA2* on light–dark hair color is smaller by an order of

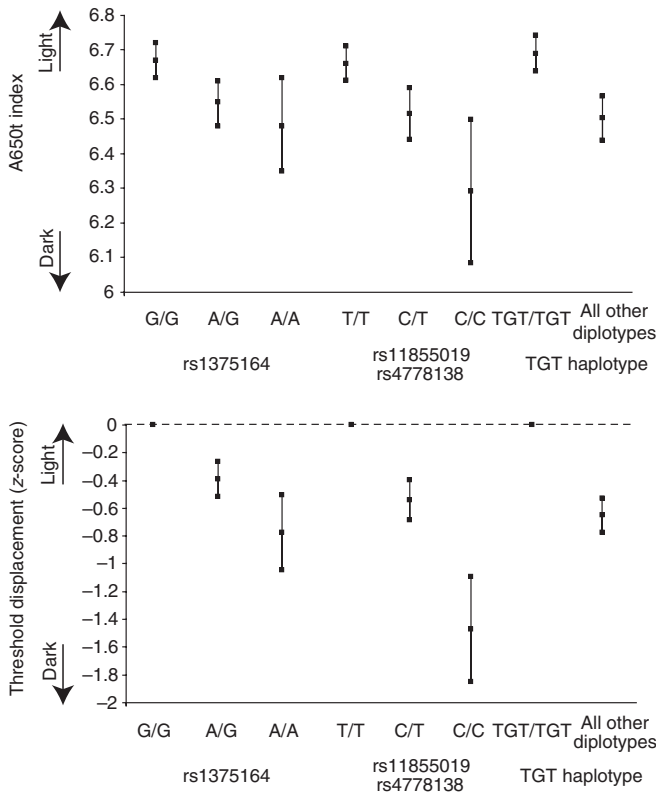

Figure 3. The effect of nucleotide polymorphisms in *OCA2* on light dark-hair color. The difference in mean A650t index (top) and thresholds between categories of hair color (bottom) between those individuals wild type at *OCA2* and those with variations at the SNPs rs1375164 and rs11855019/rs4778138 in addition to the TGT (blue eye) diplotype used by Duffy *et al.* (2007).

magnitude than its effect on eye color. Where others have found the rs12913832 and rs1129038 SNPs of the *OCA2–HERC2* locus explained much of eye color (Eiberg *et al.*, 2008; Sturm *et al.*, 2008), we have found those variants to explain even less variation in spectrophotometrically approximated eumelanin than either rs4778138 or rs1275164 in *OCA2*.

The analyses presented do not confirm the utility of spectrophotometric estimation of eumelanin compared with that of ordered ratings of hair color. Further investigation, including replication, will be required to determine the complement of genes influencing hair color and in the process determine whether spectrophotometry has any benefit over ordered ratings.

MATERIALS AND METHODS

Samples

The data for this study were collected as part of a longitudinal study investigating the development of melanoma risk factors. Adolescent twins were sought through various avenues around South East Queensland, Australia. Participation was conditional upon the informed consent of the twins and their parents. Details of the clinical protocol are described by McGregor *et al.* (1999) and Zhu *et al.* (1999). Approval to undertake this study was granted by the Human Research Ethics Committee of the Queensland Institute of Medical Research. The experiments were conducted in accordance with protocols in the Declaration of Helsinki Principles.

Quantifying light–dark hair color

Twins and their siblings were put through the protocol when the twins were approximately 12 and 14 years of age. At each visit, the nurse classified each participant's hair into one of the five categories, namely fair/blonde, light brown, red/auburn, dark brown, or black. A sample of hair between 2 and 5 cm was collected from the nape of the neck. If an individual had short hair (predominantly males), a sample was taken from the crown and placed in a sealable plastic ziplock bag. A sample was not taken if the hair was too short or dyed. The intensity of light reflected between 340 and 1,026 nm was captured by one of us (TF) using a UV-visible wavelength Ocean Optics USB2000 spectrophotometer (Ocean Optics Inc, Dunedin, FL). There were 1,730 individuals for whom hair color was categorized and reflectance curves were recorded. Details of measurement procedures can be found in Shekar *et al.* (2008).

The light–dark measurement of hair captured at this wavelength is expected to approximate eumelanin content. Individuals with red hair will absorb light at 650 nm and therefore confound an approximation of eumelanin. Those individuals classified as having red/auburn hair (4.6% of sample) were removed from the linkage analysis of the A650t index (Shekar *et al.*, 2008).

Ratings of hair color, taken 6 months apart, correlated 0.966 (SE ~ 0.012). The intraclass correlation for the A650t index between these two measurement events is 0.814 (0.480, 0.985).

Genotyping

Two major genome scans of parents, twins, and siblings were carried out at the Australian Genome Research Facility (AGRF), Melbourne (Ewen *et al.*, 2000) and the Center for Inherited Disease Research (CIDR), Baltimore (Weeks *et al.*, 2002). The intercalated genome scans resulted in a 796 marker map with an average intermarker spacing of 4.8 cM in Kosambi units (Zhu *et al.*, 2004). The data were cleaned for incorrect relationships between individuals, Mendelian inconsistencies, and improbable double recombinants as detailed elsewhere (Zhu *et al.*, 2004). Approximately 36% of individuals were genotyped in both the CIDR and AGRF scans, these being mainly from families without genotyped parents; between 211 and 791 markers were genotyped per individual (Zhu *et al.*, 2004).

Identity by descent estimation

Multipoint identity by descent (IBD) was estimated at each of the 761 autosomal markers for all genotyped siblings using Merlin 1.0.1 (Abecasis *et al.*, 2002), which estimates marker allele frequencies from the observed sample. To more accurately estimate IBD between siblings, 81% of parents were genotyped (but not phenotyped). The genetic map (Duffy, 2006) was based on that of Kong *et al.* (2004). The removal of those with red hair reduced the number of informative families by 77. The linkage analysis of the A650t index was performed on 298 informative families, yielding 439 informative, sibling–sibling relationships, or quasi-independent sibling pairs (Table 2).

We used maximum likelihood variance components estimation to decompose variation in the A650t index into environmental and genetic sources with the latter including variance due to a hypothetical QTL, located at each of the autosomal markers, in turn.

Table 2. The number families in the linkage analysis of the A650t index according to the number of siblings and parental genotype information after the removal of those individuals with red hair

	Number of parents genotyped			
	0	1	2	Total families
Monozygotic twins, no siblings[1]	7	2	22	31
Monozygotic twins with one sibling	1	4	21	26
Monozygotic twins with two siblings	0	1	5	6
Dizygotic twins[2]	17	38	157	212
Dizygotic twins with one sibling[3]	0	8	39	47
Dizygotic twins with two siblings	1	2	4	7
	26	55	248	329

[1]These twins are not informative for linkage but are included in the analysis to assist in the resolution of additive genetic (A) and common environmental (C) influences.
[2]Seven of these families contain one twin individual with a sibling.
[3]Two of these families contains one twin individual and two siblings.

Covariance between siblings for a linked QTL, Q, is conditioned by $\hat{\pi}$, which is the proportion of alleles shared IBD at the trait locus between siblings (Sham, 1998): $\hat{\pi} = 1/2\Pr[\text{IBD1}] + \Pr[\text{IBD2}]$. The path coefficient for the additive genetic QTL effect (q) is a function of the recombination fraction between the marker and the trait locus as well as the magnitude of the genetic influence at that trait locus. Residual additive genetic influence (a) is modeled such that the expected phenotypic covariance between siblings and dizygotic twins is $\hat{\pi}q^2 + 1/2a^2$, where $\hat{\pi}$ for each pair is calculated from the IBD values estimated in Merlin (Posthuma *et al.*, 2003). The linkage analysis was based on the model that was best able to explain the means and variances of light–dark hair color (Shekar *et al.*, 2008). We performed linkage analysis for both the quantitative A650t index and the latent trait underlying the ordered ratings of hair color.

The computer program Mx (version 1.61e Neale *et al.*, 2002) was used to estimate parameters. The test for linkage at a particular marker is the statistical significance of q^2. The difference in log likelihoods between the model where q^2 is free and the model where q^2 is zero is distributed as a 1/2:1/2 mixture of χ^2_1 and a point mass at zero and designated $\chi^2_{0,1}$ (Self and Liang, 1987). Dividing the difference in log likelihoods by 2ln10 ($\approx$4.6) produces a LOD score equivalent to that for parametric linkage analysis (Williams and Blangero, 1999). The single-point IBD for a single marker in the major pseudoautosomal region between chromosome X and Y was also estimated. The computer program MINX (Merlin in X, Abecasis *et al.*, 2002) was used to perform variance components linkage analysis for 31 markers on the X chromosome using the A650t index.

This study considered LOD scores of 3.3 and 1.9 as significant and suggestive evidence of linkage between QTL and the tested marker, respectively (Lander and Kruglyak, 1995).

Genotyping *OCA2* and *HERC2*

The complete process of selecting SNPs to genotype along the *OCA2* gene as well as the process of genotyping these SNPs is detailed in Duffy *et al.* (2007). SNPs were typed using iPLEX chemistry on a compact matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) Mass Spectrometer (Sequenom Inc, San Diego, CA) using standard methods (Zhao *et al.*, 2006). Of the 75 SNPs genotyped, some 17 were not used in this analysis either because of genotyping error or because they were monomorphic.

Subsequently, SNPs in *HERC2* were genotyped. Methods are outlined in Sturm *et al.* (2008). The rs1129038 and rs12913832 SNPs were selected for association analysis with the A650t index due to their association with eye color.

Association analyses

Association analyses between the A650t index and the SNPs in *OCA2* and *HERC2* were performed using the computer program QTDT (Abecasis *et al.*, 2000). This program implements the Quantitative Trait Disequilibrium Test to test whether there is a greater concordance between genotype and phenotype than can be expected by chance. The association analysis was performed on a background model that allowed for variance in the trait to be decomposed into additive genetic, common environmental, unique environmental, and linked QTL components (that is an ACEQ model). There were 1,441 individuals with *OCA2* SNP genotype and A650t index information for association analysis. To correct for multiple testing using the Bonferroni method would be too conservative when considering the relatedness of these SNPs (Nyholt, 2004). Using the SNP spectral decomposition program (http://genepi.qimr.edu.au/general/daleN/SNPSpD/), we determined that there were the equivalent of 38 independent tests and hence all corrections for multiple testing used this value.

CONFLICT OF INTEREST

Dr Frudakis is a shareholder of DNA Print Genomics Inc, and is its chief scientific officer.

ACKNOWLEDGMENTS

This work was supported by NHMRC Grants 950998, 981339, 241944 (to NGM), and NIH Grant CA88363. We thank Ann Eldridge and Marlene Grace for sample collection; TF for generating the spectrophotometric curves; Anjali Henders, Megan Campbell, and the staff of the Molecular Epidemiology

Laboratory for blood processing and DNA extraction; and Gu Zhu, Sarah Medland, and Allan McRae for their assistance with data analysis and useful comments. We also thank the twins as well as their parents and siblings for their participation.

SUPPLEMENTARY MATERIAL

Figure S1. Linkage analysis of hair reflectance at 650 nm (A650t index) for males and females and analysis of ordered, rated, hair color.

REFERENCES

Abecasis GR, Cardon LR, Cookson WO (2000) A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 66:279–92

Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002) Merlin—rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 30:97–101

Anderson MG, Smith RS, Hawes NL, Zabaleta A, Chang B, Wiggs JL *et al.* (2002) Mutations in genes encoding melanosomal proteins cause pigmentary glaucoma in DBA/2J mice. *Nat Genet* 30:81–5

Barrat FJ, Auloge L, Pastural E, Lagelouse RD, Vilmer E, Cant AJ *et al.* (1996) Genetic and physical mapping of the Chediak–Higashi syndrome on chromosome 1q42–43. *Am J Hum Genet* 59:625–32

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–5

Davenport GC, Davenport CB (1909) Heredity of hair color in man. *The American Naturalist* 43:193–211

Devlin B, Roeder K, Wasserman L (2001) Genomic control, a new approach to genetic-based association studies. *Theor Popul Biol* 60:155–66

Duffy DL (2006) An integrated genetic map for linkage analysis. *Behav Genet* 36:4–6

Duffy DL, Montgomery GW, Chen W, Zhao ZZ, Le L, James MR *et al.* (2007) A three-single-nucleotide polymorphism haplotype in intron 1 of *OCA2* explains most human eye-color variation. *Am J Hum Genet* 80:241–52

Eiberg H, Mohr J (1996) Assignment of genes coding for brown eye colour (BEY2) and brown hair colour (HCL3) on chromosome 15q. *Eur J Hum Genet* 4:237–41

Eiberg H, Troelsen J, Nielsen M, Mikkelsen A, Mengel-From J, Kjaer KW *et al.* (2008) Blue eye color in humans may be caused by a perfectly associated founder mutation in a regulatory element located within the *HERC2* gene inhibiting *OCA2* expression. *Hum Genet* 123: 177–87

Ewen KR, Bahlo M, Treloar SA, Levinson DF, Mowry B, Barlow JW *et al.* (2000) Identification and analysis of error types in high-throughput genotyping. *Am J Hum Genet* 67:727–36

Frudakis T, Terravainen T, Thomas M (2007) Multilocus *OCA2* genotypes specify human iris colors. *Hum Genet* 122:311–26

Frudakis T, Thomas M, Gaskin Z, Venkateswarlu K, Chandra KS, Ginjupalli S *et al.* (2003) Sequences associated with human iris pigmentation. *Genetics* 165:2071–83

Hanna BL (1956) Colorimetric estimation of the pigment concentration in hair of various color grades. *Am J Phys Anthropol* 14:153–73

Harrison GA, Owen JJ (1964) Studies on the inheritance of human skin colour. *Ann Hum Genet* 28:27–37

Ito S, Wakamatsu K, Ozeki H (2000) Chemical analysis of melanins and its application to the study of the regulation of melanogenesis. *Pigment Cell Res* 13(Suppl 8):103–9

Jackson IJ (1997) Homologous pigmentation mutations in human, mouse and other model organisms. *Hum Mol Genet* 6:1613–24

Jannot AS, Meziani R, Bertrand G, Gerard B, Descamps V, Archimbaud A *et al.* (2005) Allele variations in the *OCA2* gene (pink-eyed-dilution locus) are associated with genetic susceptibility to melanoma. *Eur J Hum Genet* 13:913–20

Kong X, Murphy K, Raj T, He C, White PS, Matise TC (2004) A combined linkage-physical map of the human genome. *Am J Hum Genet* 75:1143–8

Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11: 241–7

Lee ST, Nicholls RD, Jong MT, Fukai K, Spritz RA (1995) Organization and sequence of the human P gene and identification of a new family of transport proteins. *Genomics* 26:354–63

Levy C, Khaled M, Fisher DE (2006) MITF: master regulator of melanocyte development and melanoma oncogene. *Trends Mol Med* 12: 406–14

McGregor B, Pfitzner J, Zhu G, Grace M, Eldridge A, Pearson J *et al.* (1999) Genetic and environmental contributions to size, color, shape, and other characteristics of melanocytic naevi in a sample of adolescent twins. *Genet Epidemiol* 16:40–53

MINXChromosome X Analyses. [http://www.sph.umich.edu/csg/abecasis/Merlin/reference.html] The University of Michigan Center for Statistical Genetics MERLIN Reference Sheet

Neale MC, Boker SM, Xie G, Maes HH (2002) *Mx: statistical modelling*, 6th edn. VCU, Richmond, VA: Department of Psychiatry

Nyholt DR (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 74:765–9

Ozeki H, Ito S, Wakamatsu K, Hirobe T (1995) Chemical characterization of hair melanins in various coat-color mutants of mice. *J Invest Dermatol* 105:361–6

Ozeki H, Ito S, Wakamatsu K, Thody AJ (1996) Spectrophotometric characterization of eumelanin and pheomelanin in hair. *Pigment Cell Res* 9:265–70

Parra EJ, Kittles RA, Shriver MD (2004) Implications of correlations between skin color and genetic ancestry for biomedical research. *Nat Genet* 36:S54–60

Posthuma D, Beem AL, de Geus EJ, van Baal GC, von Hjelmborg JB, Iachine I *et al.* (2003) Theory and practice in quantitative genetics. *Twin Res* 6:361–76

Posthuma D, Visscher PM, Willemsen G, Zhu G, Martin NG, Slagboom PE *et al.* (2006) Replicated linkage for eye color on 15q using comparative ratings of sibling pairs. *Behav Genet* 36:12–7

Rebbeck TR, Kanetsky PA, Walker AH, Holmes R, Halpern AC, Schuchter LM *et al.* (2002) P gene as an inherited biomarker of human eye color. *Cancer Epidemiol Biomarkers Prev* 11:782–4

Self SG, Liang K (1987) Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. *J Am Stat Assoc* 82:605–10

Sham PC (1998) *Statistics in human genetics*. Hodder Arnold: London

Shekar SN, Duffy DL, Frudakis T, Montgomery GW, James MR, Sturm RA *et al.* (2008) Spectrophotometric methods of quantifying pigmentation human hair-influence of MC1R genotype and environment. *Photochem Photobiol* 84:719–26

Shriver MD, Parra EJ (2000) Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry. *Am J Phys Anthropol* 112:17–27

Shriver MD, Parra EJ, Dios S, Bonilla C, Norton H, Jovel C *et al.* (2003) Skin pigmentation, biogeographical ancestry and admixture mapping. *Hum Genet* 112:387–99

Sturm RA, Duffy DL, Zhao ZZ, Leite FP, Stark MS, Hayward NK *et al.* (2008) A single SNP in an evolutionary conserved region within Intron 86 of the *HERC2* gene determines human blue-brown eye color. *Am J Hum Genet* 82:424–31

Sulem P, Gudbjartsson DF, Stacey SN, Helgason A, Rafnar T, Magnusson KP *et al.* (2007) Genetic determinants of hair, eye and skin pigmentation in Europeans. *Nat Genet* 39:1443–52

Theos AC, Truschel ST, Raposo G, Marks MS (2005) The Silver locus product Pmel17/gp100/Silv/ME20: controversial in name and in function. *Pigment Cell Res* 18:322–36

Wakamatsu K, Ito S (2002) Advanced chemical methods in melanin determination. *Pigment Cell Res* 15:174–83

Weeks DE, Conley YP, Ferrell RE, Mah TS, Gorin MB (2002) A tale of two genotypes: consistency between two high-throughput genotyping centers. *Genome Res* 12:430–5

Williams JT, Blangero J (1999) Comparison of variance components and sibpair-based approaches to quantitative trait linkage analysis in unselected samples. *Genet Epidemiol* 16:113–34

Zhao ZZ, Nyholt DR, Le L, Martin NG, James MR, Treloar SA *et al.* (2006) KRAS variation and risk of endometriosis. *Mol Hum Reprod* 12:671–6

Zhu G, Duffy DL, Eldridge A, Grace M, Mayne C, O'Gorman L *et al.* (1999) A major quantitative-trait locus for mole density is linked to the familial melanoma gene CDKN2A: a maximum-likelihood combined linkage and association analysis in twins and their sibs. *Am J Hum Genet* 65:483–92

Zhu G, Evans DM, Duffy DL, Montgomery GW, Medland SE, Gillespie NA *et al.* (2004) A genome scan for eye color in 502 twin families: most variation is due to a QTL on chromosome 15q. *Twin Res* 7:197–210