

Genetic determinants of hair, eye and skin pigmentation in Europeans

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Hair, skin and eye colors are highly heritable and visible traits in humans. We carried out a genome-wide association scan for variants associated with hair and eye pigmentation, skin sensitivity to sun and freckling among 2,986 Icelanders. We then tested the most closely associated SNPs from six regions—four not previously implicated in the normal variation of human pigmentation—and replicated their association in a second sample of 2,718 Icelanders and a sample of 1,214 Dutch. The SNPs from all six regions met the criteria for genome-wide significance. A variant in *SLC24A4* is associated with eye and hair color, a variant near *KITLG* is associated with hair color, two coding variants in *TYR* are associated with eye color and freckles, and a variant on 6p25.3 is associated with freckles. The fifth region provided refinements to a previously reported association in *OCA2*, and the sixth encompasses previously described variants in *MC1R*.

Hair, eye and skin pigmentation are among the most visible examples of human phenotypic variation, with a broad normal range that is subject to substantial geographic stratification. In the case of skin, individuals tend to have lighter pigmentation with increasing distance from the equator¹. By contrast, the majority of variation in human eye and hair color is found among individuals of European ancestry, with most other human populations fixed for brown eyes and black hair.

Pigmentation in human tissue is attributable to the number, type and cellular distribution of melanosomes (subcellular compartments produced by melanocytes that synthesize and store the light-absorbing polymer melanin)^{2–4}. Variation in pigmentation among individuals is thought to be caused by biochemical differences that affect the number of melanosomes produced, the type of melanin synthesized (either the black-brown colored eumelanin or the red-yellow colored pheomelanin) and the size and shape of the melanosomes.

The key physiological role of skin pigmentation seems to be to absorb ultraviolet radiation (UVR). Dark pigmentation reduces UVR-induced photolysis of folate and protects skin cells from exposure to UVR, which can cause sunburn and increase the risk of skin cancer³. However, this protective role must be weighted against the reduced amount of UVR available for the synthesis of vitamin D3. It is generally believed that the geographic distribution

of human skin pigmentation today reflects a history of adaptation to latitude-dependent levels of UVR. Notably, there is no known physiological role for hair and eye color.

It has long been recognized that pigmentation differences between individuals are due to inherited traits^{5–10}. According to the list of mouse coat color genes produced by the International Albinism Center (see URL listed in Methods below), mutations in 127 genes are known to affect pigmentation in mice, among which 68 genes have human homologs and thus represent promising candidates for the genes that account for differences in human pigmentation¹¹. Of these 68 genes, 29 have been associated with rare pigmentation anomalies and syndromes, such as albinism and Hermansky-Pudlak syndrome. Common variants that are associated with normal pigmentation variation in humans have only been identified in six genes. Several coding variants in *MC1R* (which encodes the melanocortin 1 receptor) are associated with red hair, fair skin, freckles, poor tanning response and higher risk of skin cancer^{12–14}. Analyses of hair and eye color have revealed strong linkage of brown eye and hair color to a region on chromosome 15 that encompasses the gene *OCA2* (encoding the pink-eye dilution, or oculocutaneous albinism II, protein)^{6,10,15}, which had previously been linked to albinism. Coding and non-coding variants in *OCA2* have since been associated with blue versus

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brown eye color, dark versus light hair and fair skin^{16–18}. Expanding on a discovery initially made in zebrafish, a coding SNP in the *SLC24A5* gene (encoding solute carrier family 24, member 5) was recently found to be associated with skin pigmentation in admixed African-American and African-Caribbean populations¹⁹. Similar gene-association approaches have been used to relate variants in *MATP* (encoding membrane-associated transporter protein, also known as *SLC45A2*)²⁰, *ASIP* (encoding agouti signaling protein, non-agouti homolog (mouse))²¹ and *TYR* (encoding tyrosinase)²² to differences in skin pigmentation among populations of mixed African and European ancestry.

Several recent evolutionary analyses have yielded signals of positive selection in different sets of pigmentation candidate genes in populations of European and East Asian ancestry, but there is little evidence for selection in populations of African ancestry^{23–26}. This supports the idea that dark pigmentation represents the ancestral state in humans and that the lighter pigmentation of European and East Asian groups can be traced to convergent selective sweeps of mutations in different genes.

With the aim of identifying variants that affect the variability of normal pigmentation in humans, we carried out a genome-wide association scan for sequence variants that influence hair color, eye color, freckles and skin sensitivity to sun using a set of 317,511 SNPs genotyped in 2,986 Icelanders. Promising SNPs were tested in replication samples of 2,718 Icelanders and 1,214 Dutch.

RESULTS

The frequencies of variants associated with natural hair and eye color, skin sensitivity and the presence of freckles in the two Icelandic samples and the Dutch sample are shown according to sex in **Table 1**. The samples are broadly similar, although the Icelanders have red hair, freckles and green eyes more often and brown eyes less

often. The most notable difference between the sexes is the higher frequency of green eyes and freckles in females. The higher frequency of green eyes in females is consistent with a previous report in which eye color was assessed by a single expert¹⁸.

We examined the association of sequence variants with pigmentation traits in six genome-wide association scans of the Icelandic discovery sample. We carried out two scans for eye color (blue versus green and blue versus brown), two for hair color (red versus non-red and blond versus brown) and two for skin pigment traits (skin sensitivity to sun and the presence of freckles). Overall, these genome scans revealed 104 associations that reached genome-wide significance ($P < 1.5 \times 10^{-7}$), accounted for by 60 distinct SNPs (**Supplementary Table 1** online), of which 32 showed genome-wide association with only one pigmentation trait, 12 with two traits and 16 with three traits. The 60 SNPs were clustered in different genomic regions along five different chromosomes (6, 12, 14, 15 and 16; **Supplementary Figs. 1–5** online), with the largest covering 1 Mb on chromosome 16 and the smallest covering a single SNP on chromosome 12. Notably, two of the regions overlap with or are near well-known pigmentation genes (*MC1R* on chromosome 16 and *OCA2* on chromosome 15), and one of the regions is near a strong candidate pigmentation gene (*KITLG* on chromosome 12, which encodes the KIT ligand). One of the remaining two regions overlaps with *SLC24A4* on chromosome 14, which belongs to the same family as *SLC24A5*, a recently discovered pigmentation gene¹⁹. The other is located between *IRF4* (encoding interferon regulatory factor 4) and *EXOC2* (also known as *SEC5L1*, and encoding exocyst complex component 2) on chromosome 6, neither of which has previously been reported to affect pigmentation.

We defined a subset of seven SNPs that capture the strongest association signals within these five regions from the Icelandic discovery sample. In addition, we chose two SNPs located in *TYR*, a key pigmentation gene on chromosome 11, that showed associations that

Table 1 Frequencies of eye, hair and skin pigmentation types among Icelandic and Dutch individuals^a

Pigmentation type	Iceland discovery group (N = 2,986)		Iceland replication group (N = 2,718)		Dutch group (N = 1,214)	
	Male (N = 911)	Female (N = 2,075)	Male (N = 1,153)	Female (N = 1,565)	Male (N = 696)	Female (N = 518)
Eye						
Blue or gray	80.0	70.3	79.6	68.2	69.5	52.3
Green	8.0	17.9	9.7	21.0	5.6	17.2
Brown or black	9.9	10.3	8.1	8.6	19.1	24.3
Other or unknown	2.1	1.5	2.6	2.2	5.7	7.4
Hair						
Red or reddish	6.1	8.1	5.9	7.6	1.9	3.3
Blond	15.3	15.2	14.7	17.4	22.1	19.7
Dark blond or light brown	50.8	48.1	53.2	45.8	50.9	50.2
Dark brown or black	26.1	26.3	23.9	28.1	25.0	26.8
Unknown	1.6	2.3	2.3	1.1	0.1	0.0
Skin sensitivity to sun^b						
Positive	29.3	35.5	29.0	34.2	36.5	46.5
Negative	66.0	58.6	66.3	59.6	63.3	53.5
Unknown	4.7	5.9	4.7	6.1	0.2	0.0
Freckles						
Present	38.4	50.8	42.8	60.3	29.3	45.2
Absent	57.3	45.4	55.5	38.3	70.1	54.1
Unknown	4.3	3.7	1.6	1.3	0.6	0.8

^aFrequencies are given in percentages. ^bBased on the Fitzpatrick score in the Icelandic samples. Estimated from related questions in the Dutch sample (see Methods).

Table 2 Association of genetic variants with eye color in the two Icelandic and one Dutch sample^a

	Locus	Variant	Iceland		Netherlands OR (95% CI)	<i>P</i>
			Discovery OR (95% CI)	Replication OR (95% CI)		
Blue versus brown eyes	<i>SLC24A4</i>	rs12896399 T	1.15 (0.95, 1.38)	1.29 (1.05, 1.59)	1.12 (0.91, 1.36)	0.032
	<i>KITLG</i>	rs12821256 C	1.13 (0.89, 1.42)	1.20 (0.92, 1.56)	0.96 (0.71, 1.30)	0.31
	6p25.3	rs1540771 A	1.11 (0.93, 1.34)	1.18 (0.95, 1.46)	1.07 (0.87, 1.30)	0.10
	<i>TYR</i>	rs1393350 A	1.20 (0.98, 1.47)	1.27 (1.01, 1.60)	1.18 (0.94, 1.48)	0.0044
		rs1042602 C	1.01 (0.83, 1.24)	0.99 (0.78, 1.25)	0.97 (0.79, 1.19)	1.00
	<i>OCA2</i>	rs1667394 A	29.43 (21.47, 40.35)	18.46 (12.93, 26.35)	15.34 (10.75, 21.88)	1.3×10^{-241}
		rs7495174 A	6.90 (3.85, 12.39)	5.56 (3.02, 10.23)	4.87 (2.43, 9.74)	3.0×10^{-24}
	<i>MC1R</i>	rs1805008 T	1.15 (0.87, 1.52)	1.02 (0.77, 1.35)	1.29 (0.88, 1.89)	0.20
		rs1805007 T	1.37 (0.98, 1.93)	0.95 (0.70, 1.28)	0.90 (0.60, 1.36)	0.044
		<i>SLC24A4</i>	rs12896399 T	2.06 (1.76, 2.42)	1.49 (1.27, 1.73)	2.08 (1.58, 2.74)
Blue versus green eyes	<i>KITLG</i>	rs12821256 C	0.92 (0.76, 1.11)	1.09 (0.90, 1.33)	1.18 (0.78, 1.80)	0.34
	6p25.3	rs1540771 A	0.99 (0.85, 1.16)	1.14 (0.98, 1.33)	0.88 (0.68, 1.15)	0.59
	<i>TYR</i>	rs1393350 A	1.52 (1.28, 1.81)	1.43 (1.21, 1.71)	1.38 (1.01, 1.89)	3.3×10^{-12}
		rs1042602 C	1.08 (0.91, 1.27)	0.88 (0.74, 1.05)	1.16 (0.88, 1.52)	0.11
	<i>OCA2</i>	rs1667394 A	6.74 (4.61, 9.83)	5.83 (4.07, 8.36)	5.96 (3.48, 10.21)	1.5×10^{-53}
		rs7495174 A	1.41 (0.75, 2.62)	2.02 (1.12, 3.65)	1.45 (0.52, 4.01)	0.11
	<i>MC1R</i>	rs1805008 T	1.04 (0.83, 1.31)	0.85 (0.69, 1.04)	0.87 (0.55, 1.37)	0.92
		rs1805007 T	0.94 (0.73, 1.22)	0.74 (0.59, 0.92)	1.12 (0.63, 1.98)	0.73

^aThe three samples consisted of 2,986 Icelandic discovery individuals, 2,718 Icelandic replication individuals and 1,214 Dutch replication individuals.

Proteins encoded by each gene: *KITLG*, KIT ligand; *MC1R*, melanocortin 1 receptor; *OCA2*, oculocutaneous albinism II; *SLC24A4*, solute carrier family 24, member 4; *TYR*, tyrosine.

were near genome-wide significance in two of the scans ($P < 6 \times 10^{-6}$; **Supplementary Fig. 6** online). No SNPs in the other candidate genes remained significant after correcting for the number of SNPs in these candidate genes, possibly owing to the lack of power. All nine SNPs showed similar strength of association with the same pigmentation traits in the Icelandic and Dutch replication samples (**Tables 2–4** and **Supplementary Table 2** online). They were also all significant in

the combined discovery and replication samples, after correction for the 317,511 SNPs tested and the six genome-wide scans carried out ($P < 2.6 \times 10^{-8}$). We summarize the primary and secondary pigmentation traits that are associated with the SNPs in these six genomic regions (**Fig. 1**) in separate sections below and discuss whether they have been subject to positive selection (**Supplementary Table 3** online).

Table 3 Association of genetic variants with hair color in the two Icelandic and one Dutch sample^a

	Locus	Variant	Iceland		Netherlands OR (95% CI)	<i>P</i>
			Discovery OR (95% CI)	Replication OR (95% CI)		
Red versus non-red hair	<i>SLC24A4</i>	rs12896399 T	1.06 (0.85, 1.31)	1.07 (0.85, 1.34)	0.88 (0.52, 1.49)	0.56
	<i>KITLG</i>	rs12821256 C	1.01 (0.78, 1.31)	0.88 (0.67, 1.17)	0.65 (0.27, 1.55)	0.84
	6p25.3	rs1540771 A	1.01 (0.82, 1.24)	1.18 (0.94, 1.48)	1.05 (0.63, 1.76)	0.88
	<i>TYR</i>	rs1393350 A	1.04 (0.83, 1.30)	1.05 (0.82, 1.34)	0.79 (0.43, 1.45)	0.81
		rs1042602 C	0.86 (0.69, 1.07)	0.98 (0.77, 1.27)	1.21 (0.71, 2.07)	0.14
	<i>OCA2</i>	rs1667394 A	0.91 (0.58, 1.44)	0.81 (0.49, 1.33)	1.44 (0.53, 3.96)	0.83
		rs7495174 A	1.49 (0.70, 3.18)	1.26 (0.58, 2.73)	1.15 (0.23, 5.73)	0.16
	<i>MC1R</i>	rs1805008 T	7.86 (5.96, 10.36)	4.53 (3.55, 5.77)	3.71 (1.85, 7.43)	4.2×10^{-95}
		rs1805007 T	12.47 (9.37, 16.60)	6.12 (4.78, 7.82)	13.02 (7.02, 24.16)	2.0×10^{-142}
		<i>SLC24A4</i>	rs12896399 T	2.56 (2.12, 3.09)	2.34 (1.94, 2.82)	1.86 (1.47, 2.36)
Blond versus brown hair	<i>KITLG</i>	rs12821256 C	2.32 (1.86, 2.89)	1.90 (1.52, 2.38)	2.43 (1.67, 3.54)	3.8×10^{-30}
	6p25.3	rs1540771 A	0.69 (0.58, 0.82)	0.85 (0.71, 1.03)	0.92 (0.73, 1.17)	1.1×10^{-7}
	<i>TYR</i>	rs1393350 A	1.29 (1.06, 1.56)	1.36 (1.12, 1.66)	1.22 (0.94, 1.59)	0.00011
		rs1042602 C	0.85 (0.70, 1.03)	0.81 (0.66, 1.00)	0.94 (0.74, 1.20)	0.021
	<i>OCA2</i>	rs1667394 A	4.94 (3.16, 7.71)	5.96 (3.73, 9.52)	5.51 (3.49, 8.69)	5.5×10^{-35}
		rs7495174 A	1.92 (0.95, 3.90)	1.84 (0.86, 3.95)	0.82 (0.40, 1.68)	0.070
	<i>MC1R</i>	rs1805008 T	1.88 (1.40, 2.51)	1.74 (1.33, 2.28)	1.93 (1.25, 2.96)	2.2×10^{-11}
		rs1805007 T	2.34 (1.69, 3.24)	2.00 (1.52, 2.64)	1.59 (0.95, 2.66)	1.9×10^{-13}

^aThe three samples consisted of 2,986 Icelandic discovery individuals, 2,718 Icelandic replication individuals and 1,214 Dutch replication individuals.

Proteins encoded by each gene: *KITLG*, KIT ligand; *MC1R*, melanocortin 1 receptor; *OCA2*, oculocutaneous albinism II; *SLC24A4*, solute carrier family 24, member 4; *TYR*, tyrosine.

Table 4 Association of genetic variants with skin sensitivity to sun and freckles in the two Icelandic and one Dutch sample^a

Locus	Variant	Iceland			Netherlands OR (95% CI)	P
		Discovery OR (95% CI)	Replication OR (95% CI)			
Skin sensitivity to sun	<i>SLC24A4</i>	rs12896399 T	1.21 (1.07, 1.36)	1.04 (0.92, 1.18)	0.98 (0.84, 1.16)	0.00035
	<i>KITLG</i>	rs12821256 C	1.07 (0.93, 1.24)	1.22 (1.05, 1.42)	0.84 (0.66, 1.08)	0.71
	6p25.3	rs1540771 A	1.21 (1.08, 1.36)	1.12 (0.99, 1.26)	1.12 (0.95, 1.32)	4.0×10^{-6}
	<i>TYR</i>	rs1393350 A	1.26 (1.11, 1.43)	1.49 (1.31, 1.70)	1.11 (0.92, 1.34)	1.6×10^{-6}
		rs1042602 C	0.96 (0.85, 1.09)	1.05 (0.91, 1.20)	0.87 (0.73, 1.02)	0.12
	<i>OCA2</i>	rs1667394 A	1.24 (0.95, 1.62)	1.24 (0.93, 1.65)	1.34 (1.00, 1.81)	0.0034
		rs7495174 A	1.30 (0.87, 1.96)	0.99 (0.64, 1.53)	1.65 (1.03, 2.63)	0.17
	<i>MC1R</i>	rs1805008 T	2.30 (1.94, 2.73)	2.07 (1.77, 2.43)	1.65 (1.23, 2.20)	1.8×10^{-43}
		rs1805007 T	2.94 (2.42, 3.58)	2.51 (2.11, 2.98)	2.01 (1.44, 2.81)	1.8×10^{-55}
		rs12896399 T	0.99 (0.88, 1.11)	1.04 (0.92, 1.16)	1.03 (0.87, 1.22)	1.00
Freckles	<i>KITLG</i>	rs12821256 C	0.89 (0.78, 1.02)	1.01 (0.88, 1.17)	0.96 (0.75, 1.24)	0.074
	6p25.3	rs1540771 A	1.40 (1.26, 1.57)	1.25 (1.11, 1.40)	1.26 (1.06, 1.49)	3.7×10^{-18}
	<i>TYR</i>	rs1393350 A	1.13 (1.00, 1.28)	1.13 (1.00, 1.28)	1.10 (0.91, 1.32)	0.0029
		rs1042602 C	1.32 (1.17, 1.49)	1.39 (1.22, 1.58)	1.23 (1.04, 1.46)	1.5×10^{-11}
	<i>OCA2</i>	rs1667394 A	1.16 (0.90, 1.48)	1.09 (0.83, 1.41)	1.39 (1.02, 1.88)	0.026
		rs7495174 A	0.84 (0.58, 1.21)	0.82 (0.55, 1.23)	1.04 (0.65, 1.66)	0.29
	<i>MC1R</i>	rs1805008 T	2.63 (2.21, 3.11)	2.49 (2.11, 2.93)	2.06 (1.54, 2.76)	2.8×10^{-60}
		rs1805007 T	4.37 (3.56, 5.37)	2.54 (2.13, 3.04)	3.96 (2.81, 5.58)	1.2×10^{-96}

^aThe three samples consisted of 2,986 Icelandic discovery individuals, 2,718 Icelandic replication individuals and 1,214 Dutch replication individuals.

Proteins encoded by each gene: *KITLG*, KIT ligand; *MC1R*, melanocortin 1 receptor; *OCA2*, oculocutaneous albinism II; *SLC24A4*, solute carrier family 24, member 4; *TYR*, tyrosine.

MC1R

A total of 38 SNPs spanning a 1-Mb region of strong linkage disequilibrium (LD) on chromosome 16 showed an association with red hair, skin sensitivity to sun and freckles that reached genome-wide significance, and they also showed a trend towards association with blond hair. The SNP rs4785763 most effectively captured the association (odds ratio (OR) = 5.62, $P = 3.2 \times 10^{-56}$ for red hair, OR = 2.03, $P = 1.2 \times 10^{-33}$ for freckles). This region contains the well-documented *MC1R* gene. More than 30 non-synonymous mutations have been described in populations of European ancestry that impair the normal function of the *MC1R* gene product^{13,14}, leading to the generation of melanosomes that contain red-yellow pheomelanin rather than brown-black eumelanin^{4,27} and resulting in pigmentation traits such as red and blond hair, freckles, fair skin and sensitivity to UVR^{12,13}. Two non-synonymous *MC1R* mutations are common enough in European populations to have a major effect on normal differences in pigmentation: R151C (rs1805007) and R160W (rs1805008)¹⁴, neither of which is assayed on the Illumina 317K SNP chip. After genotyping these SNPs in the Icelandic and Dutch samples, we found that their T alleles (that is, the mutated alleles) were correlated with the A allele of rs4785763 and that the strong association with rs4785763 disappeared in both samples when adjusted for rs1805007 and rs1805008. We therefore concluded that the association signal detected in the genome scan is due to the previously documented non-synonymous mutations in *MC1R*.

The T alleles of rs1805007 and rs1805008 are found at a frequency of 0.142 and 0.108, respectively, in the CEPH Utah (CEU) HapMap sample, but are not present in the East Asian (ASN) and Nigerian Yoruban (YRI) HapMap samples²⁸. Although this represents only a moderate level of population divergence and is not consistent with strong selective sweep on these variants in European populations, we note that only 5.13% of HapMap SNPs that have the same overall frequency in the CEU and ASN samples show a greater difference between these populations. Moreover, only 6.6% and 6.2% of the

alleles with equal frequencies in the CEU sample have greater extended haplotype homozygosity (based on the iREHH (integrated relative extended haplotype homozygosity) statistic; see Methods) than the rs1805007 and rs1805008 T alleles, respectively. These findings suggest that both mutated alleles may have been at least weakly affected by recent positive selection.

6p25.3

Two SNPs that lie only 8 kb apart in region 6p25.3, rs4959270 and rs1540771, showed association with the presence of freckles in the Icelandic sample that reached genome-wide significance (**Supplementary Table 1**). This small segment lies between two genes, *SEC5L1* and *IRF4*, neither of which is an obvious pigmentation candidate gene; no such genes are found within the LD range of the two SNPs. Although strongly correlated ($r^2 = 0.77$), the A allele of rs1540771 had the stronger association (OR = 1.40, $P = 1.9 \times 10^{-9}$) and remained significant after adjusting for rs4959270 ($P = 0.043$), whereas the association of rs495270, adjusted for rs1540771, did not remain significant ($P = 0.34$). The association of rs1540771 with freckles was confirmed in the Icelandic and Dutch replication samples (**Table 4**). Notably, the A allele of rs1540771 shows secondary associations with brown (rather than blond) hair and with skin that is sensitive to UVR (**Fig. 1** and **Tables 3** and **4**). Thus, like *MC1R*, the variant on 6p25.3 that is associated with freckles is also associated with sun sensitivity, but unlike *MC1R*, it shows no association with red hair.

The frequency of the rs1540771 A allele is approximately 50% in European populations, but 30% and 5% in the East Asian and YRI HapMap samples, respectively (6.3% of HapMap SNPs of a similar frequency in the CEU and YRI HapMap samples differ more in frequency), and only 4.1% of alleles at the same frequency in the CEU HapMap data set have greater iREHH values. This suggests that the rs1540771 A allele has been subject to positive selection in European populations, perhaps owing to its effect on reduced skin pigmentation. In addition, SNPs that are near rs1540771 were recently

shown to be among the SNPs most strongly associated with geographic location in a British population²⁹.

Tyrosinase

The two SNPs in *TYR* chosen for replication, rs1042602 and rs1393350, are found in the same block of LD ($r^2 = 0.16$ in the Icelandic sample), but their pigmentation association effects are essentially independent. The association of rs1042602 (a non-synonymous S192Y mutation) with freckles in the Icelandic discovery sample was close to reaching genome-wide significance (OR = 1.32, $P = 5.3 \times 10^{-6}$), which was confirmed in the replication samples (combined $P = 1.5 \times 10^{-11}$; **Table 4**). Although previous studies have reported that this SNP may be associated with skin²² and eye color¹⁷, we did not detect an association with any of the pigmentation traits studied other than freckles. This sets rs1042602 apart from the variants in *MC1R* and 6p25.3, where the association with freckles is accompanied by an association with sun sensitivity and hair color (**Fig. 1**). The ancestral C allele of rs1042602 is fixed in the East Asian and YRI HapMap samples, whereas the A allele is found at a frequency of approximately 35% in European populations. There is strong evidence that the rs1042602 A allele (which is associated with the absence of freckles) has been subject to positive selection in European populations (**Supplementary Table 3**). Thus, only 1.7% of comparable HapMap SNPs show greater frequency differences between the CEU and YRI samples and only 0.37% show greater frequency differences between the CEU and East Asian samples. Moreover, only 0.55% of alleles of the same frequency in the HapMap CEU samples have greater or equal *irEHH* values.

The second SNP in *TYR* chosen for replication, rs1393350, is strongly correlated with the rs1126809 SNP, which codes for a non-synonymous R402Q mutation ($D' = 1$ and $r^2 = 0.86$). The association of the A allele of rs1393350 with blue versus green eye color was close to reaching genome-wide significance (OR = 1.52, $P = 2.0 \times 10^{-6}$), which was confirmed in the replication samples (combined $P = 3.3 \times 10^{-12}$; **Table 2**). For this SNP, the greatest difference in allele frequency was between blue- and green-eyed individuals, with brown-eyed individuals having an intermediate frequency (**Fig. 1**). In addition to the primary association with eye color, possible secondary associations with blond versus brown hair and with skin sensitivity to sun were also detected (**Tables 3 and 4**). However, despite the pleiotropic effect of rs1393350 on pigmentation traits, we found no evidence for the action of positive selection based on population divergence or extended haplotype homozygosity.

SLC24A4

Three SNPs (rs4904864, rs4904868 and rs2402130) in a 37-kb region on chromosome 14 showed association with blond versus brown hair and blue versus green eyes in the Icelandic discovery sample that reached genome-wide significance (all $P < 1.9 \times 10^{-8}$, **Supplementary Table 1**). This region is located within a single block of LD that contains the first exons of *SLC24A4*. This is the first report of any link

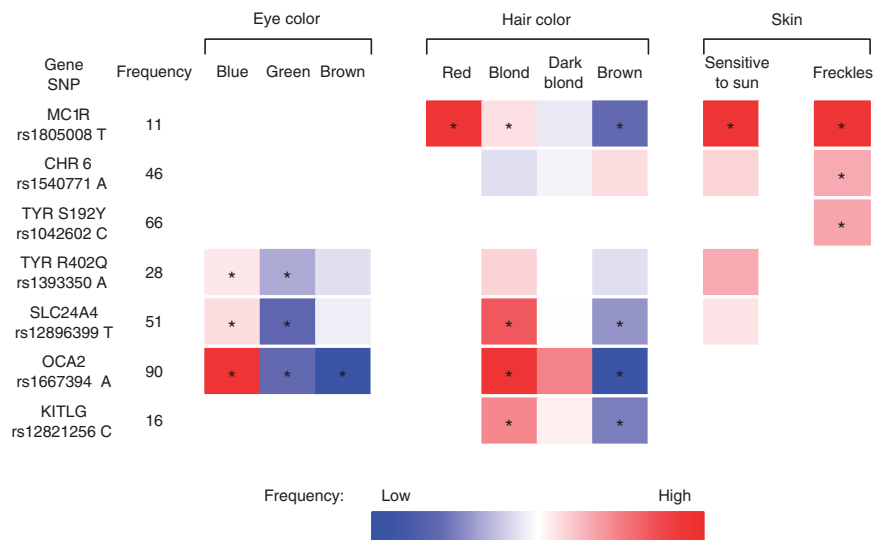


Figure 1 A schematic representation of how different genetic variants are associated with pigmentation. For eye and hair color, each cell shows how frequent the genetic variant is for each phenotype relative to the population frequency of the variant. For sun sensitivity and freckles, each cell shows how frequent the variant is in this group compared with people who are not sensitive to sun or have not had freckles, respectively. The OR scale is used to compare frequencies. For simplicity, only cells that correspond to characteristics with significant association ($P < 0.001$) are colored. Cells that correspond to highly significant results from the six genome-wide scans are marked with an asterisk ($P < 1 \times 10^{-8}$). For simplicity, only one variant is shown for each of the *MC1R* and *OCA2* genes, as the other variants have different association profiles for both genes. CHR, chromosome. Proteins encoded by each gene: *KITLG*, KIT ligand; *SLC24A4*, solute carrier family 24, member 4; *TYR*, tyrosinase.

between pigmentation and variants in *SLC24A4*, but the gene belongs to the same family as *SLC24A5*, which is a recently discovered pigmentation gene¹⁹. No common SNPs at *SLC24A5* are available in our data set; all SNPs in the region have a frequency of less than 1%.

Analysis of two SNP haplotypes from the Illumina 317K chip within the block of LD revealed that the haplotypic combination of the rs4904868 C allele and the rs2402130 A allele had a stronger and more significant association with pigmentation traits than any of the three individual SNPs in the Icelandic discovery sample (OR = 2.56, $P = 8.5 \times 10^{-24}$ for blond versus brown hair and OR = 2.06, $P = 2.0 \times 10^{-18}$ for blue versus green eyes). This haplotype almost completely accounted for the association signal provided by the three SNPs individually, with adjusted association P values that were greater than 0.25, except for the association of rs4904868 with blond versus brown hair ($P = 0.032$). An analysis of the HapMap data revealed that the haplotype tags ($r^2 = 1$) a group of equivalent SNP alleles (rs12896399 T allele, rs4904866 T allele, rs1885194 C allele and rs17184180 A allele) that have a frequency of 60% in the CEU sample but less than 1% in the YRI sample. The T allele of rs12896399 showed a similarly strong association with blond versus brown hair and blue versus green eyes in the Icelandic and Dutch replication samples as in the Icelandic discovery sample (**Tables 2 and 3**).

The high frequency of the rs12896399 T allele in the CEU HapMap sample relative to the frequency in the YRI HapMap sample (2.1% of autosomal SNPs in HapMap show a greater difference in frequency) and the low diversity of CEU haplotypes that carry this allele (6.4% of alleles found at 60% frequency in the CEU sample have greater *irEHH* values) suggest that it may have been influenced by positive selection in European populations.

Note that in the Icelandic and Dutch samples, the greatest difference in allele frequency for rs12896399 was between blue- and

green-eyed individuals, which is similar to what was observed for the second *TYR* variant (Fig. 1).

OCA2

A total of 16 SNPs, spanning 1 Mb on chromosome 15, showed association with blue versus brown eyes, blue versus green eyes, blond versus brown hair or some combination of these traits in the Icelandic sample that reached genome-wide significance (Supplementary Table 1). This region overlaps with the well-known gene *OCA2*, rare mutations of which have long been known to be a major cause of albinism^{16,17}. A recent study reported three common variants in intron 1 of *OCA2* (rs7495174, rs6497268 and rs11855019), which are strongly associated with eye, hair and skin pigmentation in populations of European ancestry¹⁸. Although all three SNPs were among the 16 detected in our genome scan, rs1667394 showed the strongest association (OR = 35.42, $P = 1.4 \times 10^{-124}$ for blue versus brown eyes, OR = 7.02, $P = 5.1 \times 10^{-25}$ for blue versus green eyes, and OR = 5.62, $P = 4.4 \times 10^{-16}$ for blond versus brown hair); this SNP is located 200 kb downstream of *OCA2*, within intron 4 of *HERC2* (encoding the protein hect domain and RLD 2). For each of the three pigmentation traits, the rs1667394 association was stronger in the Icelandic discovery sample than the individual association of the three previously reported SNPs. Furthermore, the rs1667394 association remained significant after adjustment for all haplotypes over the other three SNPs. However, as the link between *OCA2* and pigmentation is well established, it seems unlikely that the association signal provided by rs1667394 was due to a functional effect on *HERC2*. Rather, it may be that sequence variation in the introns of *HERC2* affects the expression of *OCA2* or possibly that as yet unidentified functional variants exist within *OCA2* that correlate with rs1667394.

The pattern of association of the rs1667394 A allele with hair and eye color was one of a gradient of reduced pigmentation, with the lowest allele frequency in brown-haired and brown-eyed individuals and the highest frequency in blond-haired and blue-eyed individuals. We note that the same kind of gradient was observed for the association of the rs1393350 A allele in *TYR* and the rs12896399 T allele in *SLC24A4* with hair color, but not with eye color (Fig. 1). It is also notable that the nominal association with skin sensitivity to sun observed in both the *TYR* and *SLC24A4* variants was not present for the *OCA2* variants, even though *OCA2* showed stronger association with both eye and hair color (Fig. 1 and Table 4).

The A allele of rs1667394 is found at a frequency of 80–90% in northern European populations. Several studies have reported that there is extremely strong positive selection acting on pigmentation, reducing the number of *OCA2* variants in populations of European ancestry^{23–25}. Similarly, we found that only 0.54% of HapMap SNPs show greater divergence than rs1667394 between the CEU and YRI samples, and 0.66% of HapMap SNPs show greater divergence between the CEU and East Asian samples. Furthermore, only 0.32% of HapMap SNPs in the CEU sample have an *irEHH* value that is greater than or equal to that observed for the rs1667394 A allele.

KITLG

A single SNP on 12q21.33, rs12821256, showed genome-wide significance in the initial scan for association with blond versus brown hair (OR = 2.32, $P = 1.9 \times 10^{-14}$). This association was confirmed in both replication samples (Table 3). The gene nearest to rs12821256 is *KITLG* (encoding the ligand for KIT receptor tyrosine kinase), a pigmentation candidate gene that has a role in controlling the migration, survival and proliferation of melanocytes³⁰. Rare mutations in the mouse homolog of *KITLG* are known to affect coat color³¹, but

no association with pigmentation has so far been reported for the human gene³⁰. This SNP lies 350 kb upstream of *KITLG* and may affect the expression of the gene, or may be in LD with a SNP that affects its expression. This idea is supported by the fact that the mouse homolog of *KITLG* is regulated by a region that is 100–300 kb upstream of the gene³⁰.

Four recent studies uncovered a strong signal suggestive of positive selection in both European and East Asian populations at *KITLG*^{23,24,26,32}. This signal stems from an extended haplotype spanning a 400-kb region that is centered on the gene and is found at frequencies of 80%, 63% and 3% in the CEU, East Asian and YRI HapMap samples, respectively. We did not find that the alleles tagging this haplotype were consistently associated with any of the six pigmentation traits. Notably, the blond hair-associated rs12821256 C allele was found almost exclusively on the background of this extended haplotype in populations of European ancestry (at a frequency of approximately 15%), but is not present in the YRI or East Asian HapMap samples. Only 1.65% of alleles at the same frequency in the CEU HapMap sample have greater or equal *irEHH* values. However, the *irEHH* value of the rs12821256 C allele was substantially reduced when examined only on the background of the extended haplotype. Thus, the rs12821256 C allele is not itself under positive selection, but rather is a hitch-hiker whose frequency is driven up by some selective advantage that is conferred by the extended haplotype.

DISCUSSION

Although numerous genes have been identified as candidates for pigmentation through animal models or linkage to diseases with mendelian patterns of inheritance, most of the genetic variants that contribute to the variability of normal human pigmentation remain unknown. Based on genome-wide association scans, we have identified several new variants that account for differences in the pigmentation of eyes, hair and skin among individuals of European ancestry. Except for the variant in 6p25.3, these variants are located within or near genes that have either been proposed by others as pigmentation candidate genes (*KITLG* and *TYR*) or have homology to known candidates (*SLC24A4*).

Each of these variants can be viewed as having a high minor allele frequency and a moderate effect on pigmentation in Europeans, with allelic ORs in the range of 1.2–2.5. This contrasts with the large effect but lower minor allele frequency of variants from the remaining two genes detected in our genome scan, *MC1R* and *OCA2*, which were described in previous reports^{12,15}. It is also fascinating to note the apparent differences in the observed association of the different variants with the pigmentation characteristics, with some variants associated with many characteristics and others with only one. Examples are the striking difference in the pattern of association with eye color for the *TYR* and *SLC24A4* variants when compared with those of *OCA2* and the difference in the sign of the correlation with blond hair color between the *MC1R* and 6p25.3 variants, both of which are associated with sensitive skin and freckles (Fig. 1). These patterns of association have an important role in creating the differences in hair, eye and skin pigmentation that are observed between individuals from European populations. Our data on the characteristics of pigmentation are based on self assessment, and it is likely that more complete and objective measurement techniques would strengthen the observed associations and potentially lead to further discoveries. In addition, the homogeneity of skin pigmentation in our discovery population reduces the power to detect new variants and to replicate previously reported variants that affect skin pigmentation (in particular those that are fixed in Icelanders).

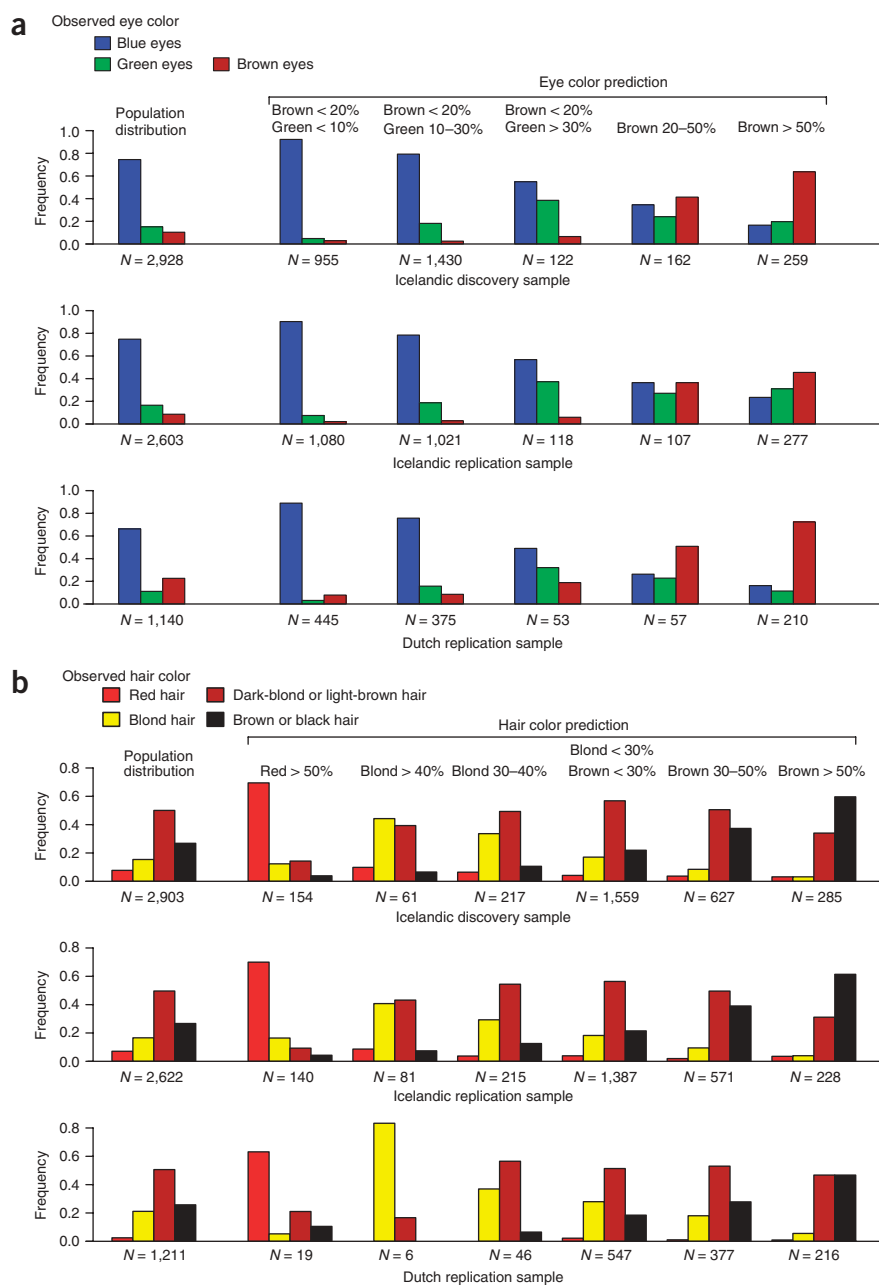


Figure 2 Overview of the accuracy of pigmentation prediction based on genotype status. (a) Eye pigmentation. (b) Hair pigmentation. The prediction rules were created from the Icelandic discovery sample and then applied to the Icelandic and Dutch replication samples. Only those individuals who were genotyped for all necessary markers, or good surrogates of these markers, were used. Histograms show the distribution of pigmentation within each sample and within groups of individuals with similar predicted pigmentation.

It has long been thought that before the migrations that first brought our species out of Africa some 60,000 years ago, ancestral human populations had characteristically dark skin, eyes and hair²⁴. This idea is consistent with the positive correlation in humans between the degree of skin pigmentation and proximity to the equator^{1,2} and with the findings that some genes that are involved in the synthesis of eumelanin are under strong purifying selection in populations who are exposed to high levels of UVR³³. More recently, several studies have provided evidence in support of the idea that positive selection drove lighter skin pigmentation to near fixation in populations at northerly latitudes, such as those of European and East Asian ancestry^{19,23–25}. Our results support this conclusion, in that most of the pigmentation variants discovered in this study show signals of positive selection in European populations. In each case the variant that is likely to contribute to lighter pigmentation of the skin has been swept to high frequency, which is consistent with positive selection on the sequence variants that undermine the formation of pigments. The most obvious functional advantage of lighter skin pigmentation in northerly latitudes is that it facilitates the synthesis of vitamin D3 in spite of low levels of UVR exposure³. However, other functional advantages or constraints cannot be ruled out.

In spite of the observed frequency difference in some of the pigmentation characteristics, we did not find evidence for a difference between the sexes in the strength of association with any of the variants (**Supplementary Table 4** online).

Given this new set of genetic determinants of pigmentation, we have attempted to predict eye and hair pigmentation based on genotype (**Fig. 2** and **Supplementary Table 5** online). For eye color, the prediction of blue versus brown eye color is dominated by variants in *OCA2*, and the discoveries described in this report add resolution to the discrimination between blue and green eye color. For hair color, the contribution of the new variants is much more substantial. Although the prediction of red hair color is based solely on *MC1R* variants, the new variants add predictive power in distinguishing the shades of non-red hair. Red and either blond or brown hair color can be excluded with a high degree of certainty for a substantial proportion of individuals.

The growing number of known sequence variants that underlie the differences in normal human pigmentation within and between populations may provide new inroads into the molecular physiology of these traits, which in turn could enhance our understanding of how they evolved. At the very least, the newly discovered genetic determinants of human pigmentation provide promising candidates for forensic geneticists and for studies of diseases of the skin and eyes that are known to be correlated with such traits.

METHODS

The Icelandic samples. A total of 2,986 Icelandic adults, recruited through cardiovascular, neoplastic, neurological and metabolic studies, were genotyped for 317,511 SNPs using the HumanHap300 BeadChip (Illumina). These studies were approved by the Data Protection Commission of Iceland and the National Bioethics Committee of Iceland. Written informed consent was obtained from all participants. Personal identifiers associated with phenotypic information

and blood samples were encrypted using a third-party encryption system as previously described³⁴. Only individuals with a genotype yield over 98% were included in the study. A second sample of 2,714 Icelandic individuals was recruited in a similar fashion and genotyped to replicate the SNPs identified in the genome-wide scan.

Each participant completed a questionnaire that included questions about natural eye color categories (blue/gray, green or black/brown), natural hair color categories (red/reddish, blond, dark blond/light brown or brown/black) and the presence of freckles at any time (Table 1). Skin sensitivity to sun was self-assessed using the Fitzpatrick skin-type score³⁵, where the lowest score (I) represents very fair skin that is very sensitive to UVR and the highest score (IV) represents dark skin that tans rather than burns in reaction to UVR exposure. Individuals scoring I and II were classified as being sensitive to sun and individuals scoring III and IV were classified as not being sensitive to sun.

No objective measurements of pigmentation (for example, spectrophotometry) were carried out. The benefits of the self-reported measurements are that they are cheap and easy to collect, but their subjective nature is likely to introduce misclassifications, leading to a loss of power in the discovery phase and a decrease in prediction accuracy.

The Dutch sample. The SNPs with the most significant associations that were identified in the genome-wide scans carried out on the Icelandic discovery sample were genotyped and tested for association in a sample of 1,214 Dutch individuals. The Dutch sample was composed of 696 males recruited for a prostate cancer study³⁶ and 518 females recruited for a breast cancer study by the Radboud University Nijmegen Medical Centre (RUNMC) and through a population-based cancer registry held by the Comprehensive Cancer Centre IKO in Nijmegen. All individuals were of self-reported European ancestry. The study protocol was approved by the Institutional Review Board of Radboud University and all study subjects gave written informed consent for the collection of questionnaire data on lifestyle, medical history and family history.

As in the case of the Icelandic samples, information about pigmentation traits for the Dutch sample was obtained through a questionnaire. The questions about natural eye and hair color were the same as those in the Icelandic questionnaire, with the addition of a category for an 'other' eye color. A total of 5.9% of the Dutch participants selected this category and were excluded from our analysis. Skin sensitivity to sun was assessed by two questions about the tendency of individuals to burn or tan when exposed to sun without sun block protection. The answers to these two questions were used to create a dichotomized grouping of individuals according to sensitivity to sun, corresponding to the grouping used for the Icelandic sample. Two questions from the Dutch questionnaire assessed the density of freckles on the face and arms, respectively. For the sake of comparison with the Icelandic data, participants reporting freckles at either location were considered as having freckles present, whereas those reporting absence of freckles at both locations were considered to have no freckles. In addition, the Dutch questionnaire included questions about skin color category (white, white with brownish tint and light-brown), the number of naevi on the left forearm and the number of serious sunburns in their lifetime.

Statistical methods. In the genome-wide association stage, Icelandic case and control samples were assayed with the Infinium HumanHap300 SNP chips (Illumina), containing 317,511 SNPs, of which 316,515 were polymorphic and satisfied our quality criteria.

A likelihood procedure described in a previous publication³⁷ was used for the association analyses. Allele-specific ORs were calculated assuming a multiplicative model³⁸. Results from multiple case-control groups were combined using a Mantel-Haenszel model³⁹. In Tables 2–4, *P* values for variants at *MC1R*, *TYR* and *OCA2* were calculated adjusting for the effect of the other variant at that locus.

Pigmentation prediction. A model to predict eye and hair pigmentation was created based on the Icelandic discovery sample (Fig. 2). A generalized linear model, in which eye color was treated as a categorical response with three categories and genotypes at all associated sequence variants were used as

covariates, was used to model eye color. A two-step model was used to predict hair color. The first step involved predicting red hair and was based solely on the *MC1R* coding variants. The second step involved modeling non-red hair color as an ordinal variable, with dark-blond or light-brown hair lying between the extremes of blond and brown or black hair. Eye and hair pigmentation in the Icelandic and Dutch replication samples were then predicted on the basis of the model parameters estimated in the Icelandic discovery sample.

Correction for relatedness and genomic control. Some of the individuals in the Icelandic case-control groups were related to each other, causing the χ^2 test statistic to have a mean > 1 and median $> 0.675^2$. We estimated the inflation factor by using a previously described procedure in which we simulated genotypes through the genealogy of 731,175 Icelanders⁴⁰. For the initial discovery samples, for which the genotypes for the 316,515 genome-wide SNPs were available, we also estimated the inflation factor by using genomic controls and calculating the average of the 316,515 χ^2 statistics and by computing the median of the 316,515 χ^2 statistics and dividing it by 0.675^2 as previously described^{41,42}. The inflation factors for the Icelandic discovery samples are given in Supplementary Table 6 online.

Single SNP and microsatellite genotyping. SNP genotyping was carried out using the Centaurus (Nanogen) platform⁴³ in (Supplementary Table 7 online). The quality of each Centaurus SNP assay was evaluated by genotyping each assay in the CEU and/or YRI HapMap samples and comparing the results with the HapMap data. Assays with mismatch rates of $> 1.5\%$ were not used, and an LD test was used for markers known to be in LD.

Controlling for population stratification. Most of the variants that show significant association with pigmentation are also present in frequencies that differ among European populations and between European, Asian and African populations. These frequency differences are to be expected given the differences in pigmentation between the populations. However, if our method of discovery were applied to a stratified sample of Europeans, without taking this stratification into account, then variants with population frequencies that correlate with pigmentation could show spurious association with pigmentation. We therefore carried out a series of tests to search for signs of stratification, even though the Icelandic population has been relatively isolated throughout its history.

First, we applied the method of genomic control to the analysis, which takes into account the genome-wide inflation of the χ^2 statistics. The inflation factors we observed were similar to inflation factors estimated from known relationships between individuals, which suggests that the overall inflation due to stratification is small (Supplementary Table 6).

Second, from a published set of 400 SNPs known to have differing frequencies between European populations⁴⁴, we selected a subset of 97 SNPs that are also present on the Illumina 317K Human Hap chip. We then tested for LD between 4,417 pairs of markers on different chromosomes among 1,984 Icelanders who are unrelated to a meiotic distance of 3. Of the 4,417 pairs tested, 225 had $P < 0.05$, compared with 220.8 expected, and 6 had $P < 0.001$, compared with 4.4 expected. We also tested for LD between the 97 SNPs and the 9 SNPs, resulting in 834 tests in which the two markers were not on the same chromosome. Again we observed no significant excess of low *P* values (39 observed compared with 41 expected at $P < 0.05$ and 2 observed compared with 0.8 expected at $P < 0.001$).

Third, the gene encoding lactase is well described and has a large degree of variation between populations⁴⁵, but no known association with pigmentation. We chose the intragenic marker rs2322659 and tested its LD with the nine SNPs that are associated with pigmentation ($P > 0.01$ in all instances). We also carried out six tests for association of rs2322659 with pigmentation without detecting any significant association.

Finally, we applied the EIGENSTRAT method⁴⁶, which relies on patterns of correlation between individuals to detect stratification, to our Icelandic discovery sample. No evidence of substantial stratification was detected, with the largest principal component estimated to explain 0.2% of the overall variation of the data. The correction factors based on correcting for the ten largest principal components are close to 1 (Supplementary Table 8

online) and do not have any effect on our conclusions. Inspection of the first few principal components suggests that they correspond to small sets of close relatives, whose relationship had not been properly accounted for.

Assessing signals of positive selection. Evidence for the effect of positive selection on the SNPs that are associated with pigmentation traits was examined by applying two methods to the data from the HapMap project (release 21; **Supplementary Table 9** online)²⁸. First, we examined whether the degree of population divergence in allele frequencies among the HapMap groups exceeded the expectations from neutral evolution. Under neutrality, the frequencies of any particular allele in a population set are shaped by the counteracting forces of genetic drift, gene flow and mutation, which constrain the expected range of allele frequency differences between populations. When the observed divergence between populations is in the upper extreme of the expected range, or outside it, the neutral model may be rejected in favor of one in which allele frequencies have been shaped by population differences in the intensity of selective forces⁴⁷.

The Wahlund F_{ST} statistic

$$F_{ST} = \frac{\text{var}(p)}{\bar{p}(1 - \bar{p})}$$

was used to measure allele frequency differences between populations, where $\text{var}(p)$ represents the variance of the frequencies of an allele from a biallelic SNP, and \bar{p} represents the average frequency of the allele among the populations under consideration. This statistic was calculated for all HapMap SNPs genotyped in at least two HapMap samples, with 3,020,798 SNPs yielding F_{ST} values based on all three HapMap samples (CEU, YRI and ASN), and 3,064,337, 3,118,875 and 3,094,443 for the population pairs CEU-YRI, CEU-ASN and YRI-ASN, respectively. For each combination of HapMap samples, the SNPs were grouped into 50 bins according to the overall frequency of the more common allele and using an interval of 0.01. To assess whether a particular SNP showed an unusual degree of population divergence, the percentile rank of each SNP's F_{ST} value was determined within each bin for each combination of HapMap samples.

The second method used to detect signals of positive selection is based on an examination of the pattern of diversity within populations. Under neutrality, there is an expected positive relationship between the frequency of an allele, its age, its variability at linked sites and the extent to which LD with other loci decays at increasing physical distance. Common alleles with unusually low diversity at linked sites and/or slow decay of LD with increasing physical distance represent probable targets of recent positive selection. We used the relative extended haplotype homozygosity (rEHH) statistic to assess the fragmentation of haplotypes around putative selected variants⁴⁸. To simplify the comparisons between different genomic regions, we calculated a single irEHH value for each allele, representing the area beneath the line defined by the rEHH point estimates that are obtained as haplotypes, extended in both directions from the allele being tested (until the EHH value in both directions has fallen below 0.05)^{49,50}. Calculations were carried out for all HapMap SNPs in the CEU HapMap sample with a minor allele frequency > 1%, yielding irEHH values for a total number of 4,906,866 alleles. To make comparisons of irEHH values meaningful between regions with different rates of recombination, the positions of SNPs were defined in centimorgans for these calculations (using recombination rate maps for phase II of the HapMap, which are available at the International HapMap website). To determine whether a particular irEHH value could be considered unusually great, thereby indicating the action of positive selection, we grouped all HapMap SNPs of the same frequency in the CEU HapMap group into separate bins and calculated the percentile rank for each irEHH value within each of the bins.

Accession codes. GenBank: *ASIP*, NM_001672; *HERC2*, NM_004667; *IRF4*, NM_002460; *KITLG*, NM_000899; *MATP* (*SLC45A2*), NM_001012509; *MC1R*, NM_002386; *OCA2*, NM_000275; *EXOC2*, NM_018303; *SLC24A4*, NM_153647; *SLC24A5*, NM_205850; *TYR*, NM_000372.

URLs. International HapMap website: <http://www.hapmap.org/>. Mouse Coat Color Genes website (maintained by W.S. Oetting and D.C. Bennett, International Albinism Center): <http://albinismdb.med.umn.edu/genes.htm>.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTION

P.S., D.F.G., A.H. and K.S. wrote the first draft of the paper. S.N.S., T.R., K.P.M., A.K., F.J., B.S., K.T., R.R., K.R.B. and J.H.O. collected the Icelandic samples and phenotypes. K.K.A. and L.A.K. collected the Dutch samples and phenotypes. S.N.S., T.R., M.J. and U.T. carried out the genotyping. P.S., D.F.G., A.H., A.M., A.P., G.T., S.S., S.P. and A.K. analyzed the data. P.S., D.F.G., S.N.S., A.H., F.J., L.A.K., J.H.O., J.G., U.T. and K.S. planned, supervised and coordinated the work. All authors contributed to the final version of the paper.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at <http://www.nature.com/naturegenetics/>.

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