Genetic Evidence for the Convergent Evolution of Light Skin in Europeans and East Asians

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Human skin pigmentation shows a strong positive correlation with ultraviolet radiation intensity, suggesting that variation in skin color is, at least partially, due to adaptation via natural selection. We investigated the evolution of pigmentation variation by testing for the presence of positive directional selection in 6 pigmentation genes using an empirical $F_{ST}$ approach, through an examination of global diversity patterns of these genes in the Centre d’Etude du Polymorphisme Humain (CEPH)-Diversity Panel, and by exploring signatures of selection in data from the International HapMap project. Additionally, we demonstrated a role for MATP in determining normal skin pigmentation variation using admixture mapping methods. Taken together (with the results of previous admixture mapping studies), these results point to the importance of several genes in shaping the pigmentation phenotype and a complex evolutionary history involving strong selection. Polymorphisms in 2 genes, ASIP and OCA2, may play a shared role in shaping light and dark pigmentation across the globe, whereas SLC24A5, MATP, and TYR have a predominant role in the evolution of light skin in Europeans but not in East Asians. These findings support a case for the recent convergent evolution of a lighter pigmentation phenotype in Europeans and East Asians.

Introduction

Skin pigmentation shows remarkable variation both within and among human populations. This variation is often explained in terms of natural (Blum 1961; Loomis 1967; Walter 1971; Branda and Eaton 1978; Kollias et al. 1991; Jablonski and Chaplin 2000) or sexual (Darwin 1871; Diamond 1992; Aoki 2002) selection. Recent work (Jablonski and Chaplin 2000; Chaplin 2004) has confirmed a strong positive correlation between skin pigmentation and ultraviolet radiation (UVR) intensity, suggesting that global variation in skin pigmentation may be the result of localized adaptation to different UVR conditions via natural selection. Although a large number of genes have been identified that explain human Mendelian pigmentation disorders and mouse coat color variability (Bennett and Lamoreux 2003), very few of these have been shown to have effects on normal variation in human skin pigmentation (Box et al. 1997; Smith et al. 1998; Flanagan et al. 2000; Kanetsky et al. 2002; Shriver et al. 2003; Bonilla et al. 2005; Graf et al. 2005; Lamason et al. 2005). Although there is strong evidence that pigmentation variation has been influenced by natural selection, it is currently unknown how selection has affected the genetic architecture of pigmentation loci in different populations, even when such populations have experienced similar levels of UVR over their evolutionary histories. For example, the dark skin that characterizes many sub-Saharan African and Island Melanesian populations may be due to shared ancestral variants or to novel genetic adaptations in the ancestral Island Melanesian population. Similarly, the light skin of Europeans and East Asians may have a common genetic origin or instead may be the result of independent adaptations to low-UVR environments.

The pigmentation candidate genes that have been identified to date have effects at various stages of the pigmentation pathway, ranging from melanogenesis, the stabilization and transport of enzymes in the melanin production pathway, the production and maintenance of melanosomes and the melanosomal environment, and the switch between the production of eumelanin and pheomelanin. In this study, we have focused on 6 of these genes. The first of these, TYR, produces the critical enzyme tyrosinase, which catalyzes the first 2 steps in the melanin synthesis pathway (Spritz 1994). A second gene in our study, MATP, has been implicated in the trafficking and intracellular processing of this critical enzyme (Costin et al. 2003). The production of melanin takes place in specialized cellular organelles known as melanosomes, and there is evidence that melanin synthesis may be dependent upon pH within these organelles (Ancans et al. 2001; Fuller et al. 2001). Puri et al. (2000) suggested that the product of the OCA2 gene, a melanosomal membrane protein, may serve as an anion transporter, thus helping to regulate melanosomal pH. More recently, it has been suggested that melanogenesis and the development of the melanosomes themselves may also be dependent on calcium levels in the melanosome, regulated by the SLC24A5 gene (Lamason et al. 2005). The final 2 genes that we examined, MC1R and ASIP, are involved in the production of the 2 types of melanin, darker eumelanin and the lighter pheomelanin. MC1R encodes for the melanocortin-1 receptor, a 7-pass transmembrane G-protein-coupled receptor that binds the hormone α-MSH. When the MC1R is activated by α-MSH, cyclic adenosine 3′,5′-monophosphate (cAMP) levels are increased and eumelanin production is stimulated through the cAMP/protein kinase A signaling pathway (Busca and Ballotti 2000). Alternatively, agouti-signaling protein, the product of ASIP, can also bind to the MC1R, blocking α-MSH and causing the production of pheomelanin rather than eumelanin. Of these 6 candidate genes, 5 (TYR, OCA2, ASIP, MC1R, and MATP) were included in our study.
and SLC24A5) have been previously associated with normal variation in skin pigmentation (Box et al. 1997; Smith et al. 1998; Schröth et al. 1999; Flanagan et al. 2000; Bastiaens et al. 2001; Shriver et al. 2003; Bonilla et al. 2005), and here we demonstrate an association for the sixth gene, MATP.

In this study, we examine the role of 6 pigmentation candidate genes in explaining global pigmentation clines primarily by looking for evidence of directional selection in patterns of variation in a geographically diverse set of populations that exhibit a range of pigmentation phenotypes. We have applied an empirical \( F_{ST} \)-based approach (Akey et al. 2002) using allele frequency data from 11,078 autosomal single nucleotide polymorphisms (SNPs) for comparison to test for population divergence in 7 different SNPs in these genes and also examined their patterns of global variation in the CEPH-Diversity Panel. We also examine several statistics that are sensitive to directional selection (locus-specific branch length [LSBL], Tajima’s \( D \); \( D_\text{n} \), \( D_\text{nRH} \)) in these and other candidate genes using data from the HapMap project and directly investigate the functional relevance of the MATP gene using an admixture mapping approach. Our results provide new insights into the genetic mechanisms underlying the human pigmentation phenotype and their evolution.

Materials and Methods

Samples

Twenty individuals each from the following populations were typed on the Affymetrix 10K whole-genome sampling assay (WGSA) Mapping Array (Santa Clara, CA): West African (Mende from Sierra Leone), Island Melanesian (Nasioi from Bougainville), South Asian (Indians from Andhra Pradesh), Native American (Nahuas from Mexico), East Asian (Chinese and Japanese from Coriel Human Cell Repository), and European (Spanish from Valencia) using the methods described in Shriver et al. (2005).

Pigmentation candidate SNPs were typed in the same 20 individuals from each population typed on the Affymetrix Arrays as well as on an average of 40 additional individuals from the same or similar populations (for a total of \( \approx 60 \) individuals per population). It was possible to sample individuals from the same regions as the populations typed on the Affymetrix Chip for the Island Melanesians (\( n = 44 \)) and Europeans (\( n = 42 \)). The additional 40 East Asian and South Asian individuals were unadmixed Chinese (\( n = 46 \)) and South Asian Indian (\( n = 45 \)) residents of Trinidad and Tobago (collected by Tamiko Brown, University of the West Indies). The additional 45 West African individuals were African-American individuals of the Gullah population of South Carolina measured to have 100% West African ancestry using 10 ancestry informative markers (AIMs) (Parra et al. 2001). The additional Native American samples comprised 14 Nahuas speakers (from the same population typed on the Affymetrix Array) and 33 Mayans (collected and kindly provided by Ken Weiss and Anne Buchanan, Penn State University).

To determine the linkage of MATP with normal variation in skin pigmentation, the C374G SNP was genotyped in 202 African-American and 122 African Caribbean individuals for whom quantitative measures of skin pigmentation as well as individual ancestry estimates were available (Shriver et al. 2003). These populations were selected because admixed populations are well suited for gene mapping due to the long-range linkage disequilibrium that is generated by admixture.

Five SNPs in these pigmentation genes that showed signals of selection in the pairwise locus-specific \( F_{ST} \) study were also typed in the full CEPH Diversity Panel (Cann et al. 2002) consisting of 53 global populations and 1059 individuals in total.

Pigmentation Gene Marker Selection and Genotyping

Pigmentation candidate SNPs were selected for inclusion in the \( F_{ST} \) portion of this study because of either previously reported allele frequency differences between populations, their location within genes believed to have an effect on pigmentation variation, or previously reported associations with normal variation in pigmentation variation. Four are nonsynonymous coding SNPs, 2 (OCA2 A355G and MC1R G314A) are synonymous, and ASIP A8818G is located in the 5’ promoter region. Each SNP was assayed using the McSNP genotyping assay (Akey et al. 2001; Ye et al. 2002). Reference SNP numbers, polymerease chain reaction, and genotyping conditions can be found in supplementary table 1 (Supplementary Material online).

\( F_{ST} \) Estimation and Percentile Rank Calculation

Unbiased estimates of Weir and Cockerham’s \( F_{ST} \) were calculated as in Akey et al. (Akey et al. 2002). We determined the rank percentile of the locus-specific \( F_{ST} \) values for each candidate SNP using the following equation:

\[
\text{Rank percentile} (x) = \frac{\text{number of loci} > \text{observed pairwise locus-specific } F_{ST}(x)}{\text{total number of loci}}.
\]

The higher the pairwise locus-specific \( F_{ST} \) value for a pigmentation SNP relative to the appropriate empirical distribution, the lower its percentile rank. SNPs for which the pairwise locus-specific \( F_{ST} \) value had a rank percentile value of 0.05 or less were classified as showing significant divergence. Thus, all \( P \) values reported for \( F_{ST} \) comparisons are empirical \( P \) values indicative of the percentile rank of the candidate SNP relative to the appropriate pairwise \( F_{ST} \) distribution. For example, the East Asian–West African pairwise \( F_{ST} \) value for the ASIP A8818G polymorphism is 0.071. This value is then compared against the empirical distribution of pairwise \( F_{ST} \) values between East Asians and West Africans calculated for the 11,078 SNPs typed on the Affymetrix Chip. Similarly, the pairwise \( F_{ST} \) value of this SNP between the European and West African samples is 0.818. This value is compared with the empirical distribution of pairwise \( F_{ST} \) estimated for Europeans and West Africans. A 1-tailed test is appropriate in this instance as we are investigating loci showing evidence of directional selection; these would fall in the upper tail of the distribution. We calculated \( P \) values using the full data set of 11,078 SNPs, as well as a subset composed of genic (\( n = 3159 \)) and nongenic (\( n = 7127 \)) SNPs. The results, regardless of which
data set is used, are qualitatively the same, with the exception of a single interpopulation comparison at TYR 192. In the full data set, the $F_{ST}$ value of 0.500 between the Europeans and West Africans has a $P$ value of 0.043. In the genic data set, the $P$ value rises to 0.057, but it remains significant in the nongenic data set ($P = 0.042$). This discrepancy might be due to the higher average $F_{ST}$ values for genic SNPs relative to nongenic SNPs (Hinds et al. 2005) or also to the smaller sample size of our genic data set.

Phylogenetic Tree Construction

Population trees were constructed using the Neighbor-Joining method (Nei and Saitou 1987) as implemented in MEGA 2.1 (Kumar et al. 2001) using average pairwise $F_{ST}$ values from the panel of 11,078 autosomal SNPs as a distance measure. Locus-specific trees were constructed in the same manner, using the pairwise locus-specific $F_{ST}$ values for each pigmentation SNP separately.

Admixture Mapping

We tested for linkage between the MATP C374G SNP and skin pigmentation in the African-American and African Caribbean samples using the program ADMIXMAP (Hoggart et al. 2003). ADMIXMAP uses a combination of Bayesian and classical approaches to model the associations of ancestry between linked marker loci and the association of a particular phenotypic trait of interest with individual admixture or ancestry at a linked marker locus. Individual ancestry estimates used in this program were based on allele frequencies at 34 AMs for West African, Native American, and European ancestry (Shriver et al. 2003). Skin pigmentation was measured using the DermaSpectrometer (Cortex Technology, Hadsund, Denmark), a narrow band reflectance spectrophotometer, following methods previously described (Shriver et al. 2003). The DermaSpectrometer estimates the concentrations of hemoglobin and melanin in the skin after the work of Diffey et al. (1984). By utilizing the differences in the absorption properties of these 2 chromophores, the DermaSpectrometer estimates the amount of skin reflectance due to melanin content of the skin and quantifies this as the $M$ (melanin) index. Higher $M$ index values indicate darker pigmentation, whereas lower $M$ index values indicate lighter pigmentation. The $M$ index values in both the African American and African Caribbean samples were normally distributed.

Analysis of Pigmentation Genes in HapMap Data

Data from a total of 3,458,541 SNPs in Release 20/Phase II of HapMap project were examined in 3 world populations: West African Yoruba (YRI), Northern Europe CEPH (CEU), and a pooled East Asian (EAS) group composed of Chinese and Japanese individuals. The genome was divided into 587,233 overlapping 25-kb sliding windows advanced by 5-kb (in other words a 20-kb overlap between adjacent windows) containing on average 29 SNPs. The position of each window was described according to the central base pair on the NCBI35 build of the human genome.

Three measures of sequence diversity indicative of the action of positive selection were calculated for each of the windows in each of the 3 populations. LSLBs and lnRH were calculated for each SNP within a window and averaged. LSLBL was calculated using the methods described in Shriver et al. (2004). When calculating lnRH, we have substituted 0.0001 for the zero value that occurs when an allele is fixed in one population (Storz et al. 2004). The Tajima’s $D$ statistic was also calculated for each window. Separate distributions were constructed for autosomal and X-chromosome windows. Windows were mapped to candidate pigmentation genes using Refseq coordinates (NCBI35 build of the human genome) of the largest transcript including an additional 10 kb of upstream and downstream sequence.

Results

Pairwise $F_{ST}$ in Pigmentation Genes

In all, 11,078 autosomal SNPs were typed, using the Affymetrix 10K Mapping Array Chip (Shriver et al. 2005), in 6 populations (representing Europeans, East Asians, Native Americans, South Asians, Island Melanesians, and West Africans) that encompass a range of geographic origins and pigment phenotypes. These formed an empirical distribution of values against which allele frequencies of 7 SNPs in 6 pigmentation candidate genes (TYR, MATP, SLC24A5, MC1R, ASIP, OCA2) in the same or similar populations (table 1) could be compared. The concept of using $F_{ST}$ distributions to detect signals of natural selection was first proposed by Lewontin and Krakauer (1973) and is based on the fact that although demographic processes will affect all regions of the genome equally, selection will act on specific loci and linked neutral variants (Cavalli-Sforza 1966). $F_{ST}$, like many statistics of genetic variation, can be sensitive to the underlying demographic history of the populations in question (Lewontin and Krakauer 1975; Nei and Maruyama 1975; Robertson 1975). As it can be very difficult to model this history accurately, comparisons of $F_{ST}$ values with empirical $F_{ST}$ distributions drawn from the same populations (which will reflect the average
demographic history) are preferable to comparisons with simulated $F_{ST}$ distributions (Black et al. 2001; Akey et al. 2002, 2004).

Five SNPs in 5 different pigmentation genes had locus-specific $F_{ST}$ values that fell within the top 5% of comparisons for at least one population pair gauged across our empirical distribution (table 2). The remaining 2 SNPs, both within the $MC1R$ gene, did not show such interpopulation partitioning of variation. SNPs with pairwise $F_{ST}$ values in the top 5% of the relevant empirical distributions were used to construct Neighbor-Joining trees as a way to graphically represent divergence among populations at these pigmentation loci (fig. 1).

Pairwise $F_{ST}$ estimates for the $ASIP$ A8818G and $OCA2$ A355G SNPs tentatively suggest a pattern of divergence between 4 populations (Europeans, East Asians, Native Americans, and South Asians) and the relatively more darkly pigmented populations of West Africa and Island Melanesia, or possibly only between West Africans and all other populations. At both loci, West Africans and Island Melanesians have higher frequencies of the ancestral alleles than the other 4 populations. Pairwise locus-specific $F_{ST}$ values falling in the top 5% of the empirical distributions are observed between West Africans and 3 other populations (South Asians, Native Americans, and Europeans) at $ASIP$ A8818G. $F_{ST}$ values between West Africans and East Asians at this locus are elevated but do not reach our cutoff value of 5% ($F_{ST} = 0.489, P = 0.065$). At $OCA2$ A355G, only West Africans and Europeans show $F_{ST}$ values falling into the top fifth percentile of relevant comparisons ($F_{ST} = 0.516, P = 0.05$). The low pairwise $F_{ST}$ values and higher frequency of ancestral alleles at both SNPs studied in these loci between West Africans and Island Melanesians hint that dark pigmentation associated with both loci in these populations may have a common evolutionary origin (Mean $F_{ST(WA/IM)} = 0.182$; $ASIP$ A8818G $F_{ST(WA/IM)} = 0.260, P = 0.282$; $OCA2$ A355G $F_{ST(WA/IM)} = 0.101, P = 0.525$).

Three loci, $TYR$ A192C, $MATP$ C374G, $SLC24A5$ A111G, show very strong signals of European-specific
divergence. High $F_{ST}$ values between Europeans and darkly pigmented populations such as West Africans and Island Melanesians are not unexpected if these genes have functional effects. However, the notably elevated pairwise $F_{ST}$ values relative to East Asians (the population in our panel that is the most similar to Europeans in pigmentation phenotype) is striking. Populations intermediate in pigmentation (Native Americans and South Asians) also exhibit $F_{ST}$ values falling in the top fifth percentile of their relevant $F_{ST}$ distributions with Europeans for these 3 loci. In the case of $SLC24A5 A111G$, South Asian pairwise $F_{ST}$ values also fall in this top fifth percentile when compared with both Europeans ($F_{ST} = 0.389, P < 0.01$) and East Asians ($F_{ST} = 0.519, P < 0.01$), but not when compared with any other population. At all 3 loci, Europeans have the highest frequency of the derived alleles relative to the other 5 populations.

Admixture Mapping of $MATP$

Four of the 5 SNPs displaying potential signals of selection ($ASIP A8818G$, $OCA2 A355G$, $TYR A192C$, and $SLC24A5 A111G$) have been previously shown to be associated with normal pigmentation variation in admixed African-American and African Caribbean populations (Shriver et al. 2003; Bonilla et al. 2005; Lamason et al. 2005). $MATP$ C374G was reported to be associated with normal variation in pigmentation in a population of European ancestry (Graf et al. 2005), but an association with variation in pigmentation between populations that differ in pigmentation levels has not been demonstrated (although observed differences in allele frequencies between such populations has led some to speculate that this may be the case [Nakayama et al. 2002; Graf et al. 2005]).

We used previously described admixture mapping techniques (McKeigue et al. 2000) to test for linkage between $MATP$ C374G genotype and quantitatively measured skin pigmentation in a sample of 202 African-American individuals. The ancestral allele, $C$, has an effect size per allele of 5 melanin units (95% CI: +2.5 to +8), and its effects are consistent with an additive mode of inheritance.

![Admixture Mapping of $MATP$](image-url)

**FIG. 1.**—Neighbor-Joining trees based on (a) average $F_{ST}$ values among the 6 populations typed on the Affymetrix 10K WGS A chip and locus-specific $F_{ST}$ values at (b) $ASIP A8818G$, (c) $OCA2 A355G$, (d) $TYR A192C$, (e) $MATP C374G$, and (f) $SLC24A5 A111G$. Populations are abbreviated as follows: WA, West African; SA, South Asian; NA, Native American; EU, European; EA, East Asian; IM, Island Melanesian.

![Distribution of $M$ index values](image-url)

**FIG. 2.**—Distribution of $M$ index values for each of the 3 genotype classes at $MATP$ C374G in a sample of 202 African-American individuals. The ancestral allele, $C$, has an effect size per allele of 5 melanin units (95% CI: +2.5 to +8), and its effects are consistent with an additive mode of inheritance.
program ADMIXMAP, we detected a significant linkage between genotype at \textit{MATP} C374G and pigmentation ($P < 0.0001$). The ancestral allele has an effect size per allele of +5 melanin units (95% confidence interval [CI]: +2.5 to +8) and is consistent with an additive rather than a dominant mode of action. We did not observe a significant association between \textit{MATP} C374G and pigmentation in the African Caribbean sample, but this may be due to both the smaller sample size and lower admixture proportions observed for this population relative to the African-American sample. Nonetheless, the 95% CIs for effect size in this population (−3 to +8) are consistent with those observed in the African-American population.

**Global Patterns of Variation in Pigmentation Genes**

To confirm and investigate further the patterns we observed in our original samples, we typed the 5 SNPs...
showing elevated $F_{ST}$ values relative to empirical distributions in 53 additional populations from the CEPH-Diversity Panel (Cann et al. 2002). Allele frequencies for each of these can be found in supplementary table 2 (Supplementary Material online), whereas figure 3A–E illustrates their global allele frequency distributions.

The pattern of diversity at ASIP 8818*G allele (the ancestral allele associated with darker pigmentation) indicates a role primarily in African/non-African divergence (sub-Saharan African frequency: 66%, all other populations: 14%) rather than between darkly and lightly pigmented populations. At OCA2 355, the derived allele (linked with lighter pigmentation) occurs at its highest frequencies across Europe and Asia but is also relatively common among Native American populations (18–34%) and is present at much lower frequencies (0–10%) among Bantu-speaking African groups. In contrast, the ancestral allele associated with dark pigmentation has a shared high frequency in sub-Saharan African and Island Melanesians. A notable exception is the relatively lightly pigmented San population of Southern Africa where the derived allele predominates (93%), although this may be simply due to small sample size ($n = 14$).

The distributions of the derived and ancestral alleles at TYR A192C, MATP C374G, and SLC24A5 A111G are consistent with the $F_{ST}$ results suggesting strong European-specific divergence at these loci. The derived allele at TYR, 192*A (previously linked with lighter pigmentation [Shriver et al. 2003]), has a frequency of 38% among European populations but a frequency of only 14% among non-Europeans. The differences between Europeans and
non-Europeans for the MATP 374*G and SLC24A5 111*A alleles (both derived alleles associated with lighter pigmentation) were even more striking (MATP European = 87%; MATP non-European = 17%; SLC24A5 European = 100%; SLC24A5 non-European = 46%). The frequency of the SLC24A5 111*A allele outside of Europe is largely accounted for by high frequencies in geographically proximate populations in northern Africa, the Middle East, and Pakistan (ranging from 62% to 100%).

Signatures of Selection in Pigmentation Genes Using HapMap Data

To supplement our analyses, we also examined the global diversity of our 6 pigmentation genes using data from the International HapMap project. Three potential indicators of directional positive selection (LSBL, Tajima’s D, and lnRH) were calculated in 25-kb overlapping windows in European (CEU), East Asian (EAS), and West African (YRI) populations separately and their significance gauged by an empirical genome-wide distribution.

The first statistic, LSBL (Shriver et al. 2004), decomposes $F_{ST}$ among 3 populations into population-specific components and provides a means to quantify the degree to which a SNP (or group of SNPs in this case) has changed in allele frequency in one population relative to the other. Second, Tajima’s D was used to summarize the allele frequency spectrum in each genomic window. Under neutrality, Tajima’s D will take on values close to zero. Significantly negative values indicate an excess of rare alleles that is consistent with recent positive directional selection or a population expansion (Tajima 1989). Although Tajima’s D is normally used in cases of full ascertainment (i.e., resequencing), previous studies have established a correlation between resequencing and dense genotyping data (Carlson et al. 2005; Voight et al. 2006). Although the HapMap data set does suffer from an ascertainment bias toward common SNPs, this should result in a skew of Tajima D values against negative values that are indicative of directional selection.

Finally, we calculated the related natural log of the ratio of heterozygosities (lnRH) between all pairwise population comparisons (Schlotterer 2002). Strongly negative values (i.e., a low ratio) indicate a reduced heterozygosity in one population relative to another and points to population-specific effect (not necessarily the case with significant Tajima D values). A simple case of strong and population-specific positive selection is expected to result in strongly negative Tajima’s D, high LSBL, and negative lnRH values. Although these are relatively simple metrics, when used together, they may provide nuanced insight in the timing and place of more complex selective events. The full set of results can be found in supplementary table 3 (Supplementary Material online).

These data confirm the unusual European-specific patterns at MATP and SLC24A5. Both genes display long range (consecutive windows) and significant indications of positive selection for all 3 statistics. In contrast, there is little evidence of a European-specific pattern in the TYR locus although the nonsynonymous TYR A192C SNP does individually show a strongly significant CEU-LSBL ($P < 0.003$) in the HapMap data as in our original findings. The contrast may be explained by the limitations of our HapMap sliding window analyses, whereby adjacent SNPs are averaged using a method that does not consider haplotype structure.

A more complex pattern of evolution is indicated in the HapMap data for the OCA2 gene. In line with the $F_{ST}$-based survey, it shows consistently strong and significant European LSBL and somewhat more erratic significance in other measures. However, it also reveals a similar if slightly weaker pattern of significance in the East Asian population consistent with our original observation of a role in lightly versus darkly pigmented
populations. Another such candidate gene, *ASIP*, also shows some tentative indications of an African-specific pattern, although this signal is weak compared with that observed for the *MATP* and *SLC24A5* genes in Europeans. The HapMap data does, however, concur with our previous finding regarding *MC1R* in failing to detect any signal of selection at this locus in any of the 3 populations.

We also examined these statistics at 28 additional pigmentation candidate loci and identified several genes showing evidence of natural selection. In particular, 2 genes (*ADAM17* and *ATRN*) showed East Asian–specific signatures comparable in strength with those observed for *MATP* and *SLC24A5* in Europeans. The *ADTB3A* gene also shows a strong and focused signature of positive selection in Africans. Several other genes showed more tentative and/or complex evidence of a selected past. These include the transcription factor *MITF*, which may have been subject to a selective sweep prior to the divergence of these 3 populations (significantly negative Tajima’s D), and *TYRP1*, which shows strong *LSB* in the European and, to a lesser extent, East Asian populations.

**Discussion**

Patterns of variation in the 6 candidate loci that we examined in this study suggest that darkly pigmented populations in West Africa and Island Melanesia may share some ancestral pigmentation alleles but that the lighter pigmentation observed in European and East Asian populations is due to independent genetic mutations in at least 3 loci: *SLC24A5*, *MATP*, and *TYR*. The initial set of populations that we examined occupied a range of different UVR environments and fell across a broad spectrum of pigmentation phenotypes. Although there is certainly variation within these populations (as well as overlap in pigmentation phenotype between some of them), 2 populations from high-UVR regions, West Africans and Island Melanesians, fall at the darker end of the pigmentation continuum, whereas 2 other populations from low-UVR regions, East Asians and Europeans, fall at the opposite end. Comparisons of genetic variation in these 2 pairs of populations should be helpful in determining if similarities in pigmentation phenotype are due to the same or independent genetic mechanisms.

Many hypotheses predict that natural selection will eliminate genetic variants associated with lighter skin in regions of high UVR as a protection against photodamage (e.g., sunburn, melanoma, and basal and squamous cell carcinomas) (Blum 1961; Kollias et al. 1991) and folic acid photodegradation (Branda and Eaton 1978; Jablonski and Chaplin 2000). The photoprotective properties of a highly melanized skin and the recent African origin of modern humans suggest that the ancestral phenotype is one of the relatively dark skin (Jablonski and Chaplin 2000; Rogers et al. 2004). If dark skin is the ancestral phenotype, then we may assume that the first migrants out of Africa were also relatively darkly pigmented.

There are 2 primary explanations for the evolution of lighter skin in regions of low UVR. The first suggests that light skin is merely due to the relaxation of functional constraint and that derived alleles associated with lighter pigmentation may have simply drifted to high frequency in the absence of strong purifying selection (Brace 1963). The second explanation suggests that in lower UVR regions, positive selection would have favored mutations leading to lighter skin as a way to maximize cutaneous vitamin D synthesis (Rana et al. 1999; Jablonski and Chaplin 2000). Given the relatively recent arrival and divergence of humans in and across Europe and Asia, the most parsimonious evolution of light skin would involve such mutations arising in a proto-Eurasian population soon after humans left Africa. Consequently, these mutations should be shared between modern Asian and European populations. Alternatively, if separate existing functional variants were driven to high frequency in East Asians and Europeans or independent de novo mutations arose and were selected in each population after the divergence of Europeans and Asians, then these would be obvious as high allele frequency differences between modern European and East Asian populations. Reduced levels of heterozygosity surrounding the *SLC24A5 A111G* polymorphism in the European, but not East Asian, HapMap populations support the latter hypothesis (Lamason et al. 2005), as do reduced polymorphism levels based on full resequencing data from *MATP* in populations of European descent (Soejima et al. 2005).

We will first address the role of selection in influencing darker pigmentation across different populations living in high-UVR environments. Current archaeological evidence suggests human presence in Island Melanesia by at least 40,000 years ago and in other parts of Sahul by at least 45,000 years ago (O’Connell and Allen 2004). If the original migrants to Oceania arrived there via a corridor of relatively high UVR, then we might expect their descendents to share ancestral pigmentation variants with African populations. However, if the ancestors of modern day Island Melanesians spent a significant amount of time in low-UVR regions prior to arriving in Oceania, then it is possible that mutations associated with lighter pigmentation could have accumulated and a readaptation to high-UVR conditions would have been necessary, leading to potential divergence between Island Melanesians and Africans at functional pigmentation loci. In actuality, both of these scenarios may apply, as we know that modern Island Melanesian populations are descended from both early migrants (arriving ~40,000 years ago) as well as later proto-Austronesian-speaking peoples from a southeast Asian homeland ~3,200 years ago (Spriggs 1997).

The discordance between our *F*<sub>ST</sub>-based divergence values and allele frequencies in the Melanesian CEPH populations at *ASIP* largely stem from the relatively low frequency of the ancestral allele in the 2 CEPH Island Melanesian populations relative to our original Island Melanesian sample. These discrepancies make it difficult to determine if *ASIP* truly underlies broad pigmentation differences between darkly and lightly pigmented populations or if instead interpolation variation at this locus can largely be explained by differences between Africans and non-Africans. This discordance between the frequencies of the *ASIP* ancestral allele in our original Island Melanesian sample and the Melanesian samples from the CEPH panel may be indicative of both the complex demographic
history of Island Melanesia (involving several migratory
events (Spriggs 1997) and probable extensive genetic drift
(Friedlaender 1975, 1987) as well as the importance of mul-
tiple loci in determining pigmentation phenotype. Some
indications of selective forces acting in the West Africans
at ASIP in the HapMap data are consistent with a role in
shaping interpopulation pigmentation, but due to the small
numbers of populations surveyed in the HapMap, these data
cannot resolve the role of ASIP across multiple darkly pig-
mented populations.

The results for OCA2 using the FST-based approach,
allele frequency distribution in the CEPH panel, and anal-
ysis of the HapMap data are largely consistent in pointing to
a role for this gene in control of light versus dark pigmen-
tation. In general, the derived allele (associated with lighter
pigmentation) is most common in Europeans and East Asians,
whereas the ancestral allele predominates in sub-
Saharan Africa and Island Melanesia. The lightly pig-
mented hunter–gatherer San population of Southern Africa
is exceptional in having a high frequency of the derived al-
lele relative to geographically proximate and more darkly
pigmented African populations (Jablonski and Chaplin
2000), further supporting the importance of OCA2 in reg-
ulating normal variation in pigmentation. The widespread
distribution of the derived allele in the CEPH-Diversity
Panel suggests that it is not necessarily a new mutation,
nor has it been restricted to a specific geographic area.
Interestingly, derived allele frequencies at this locus are
quite different between Native American (15%) and East
Asian populations (45%), suggesting that perhaps the de-
derived allele at this locus did not reach very high frequen-
cies in East Asians until after the colonization of the Americas.
Evidence from a suite of selection statistics in HapMap pop-
ulations suggests that derived allele may have been selected
in both East Asians and Europeans, and consequently a pro-
portion of the light skin phenotype in both groups may be
explained by the same genetic mechanism.

Although our data do not allow us to draw very strong
conclusions regarding the evolution of dark pigmentation in
human populations, they do provide us with compelling ev-
idence that light skin has evolved independently in Euro-
pean and East Asian populations. The strong signal of
selection in the SLC24A5 and MATP genes, and to a lesser
extent in the TYR gene, supports the active role of selection
in shaping the patterns of diversity at these loci. Linkage
between TYR and SLC24A5 and pigmentation phenotype
have been previously demonstrated (Shriver et al. 2003;
Lamason et al. 2005). Using similar methods, we observe
a strong linkage between MATP C374G and pigmentation
in African-Americans, indicating the functional relevance
of this SNP and the potentially important role that it plays
in determining interpopulation phenotypic variation. We
conclude that light pigmentation in Europeans is at least
partially due to the effects of positive directional and/or sex-
ual selection and not simply the relaxation of functional
constraint. This is consistent with a recent resequencing
study that examined variation in MATP (Soejima et al.
2005) in a global sample. The virtual absence of MATP
374G–derived allele in the sub-Saharan African popu-
lations that we examined in the CEPH-Diversity Panel is con-
sistent with the origin of this mutation outside of Africa
after the divergence of modern Asians and Europeans. In
contrast, the SLC24A5 111*A–derived allele is found at
low frequencies in several sub-Saharan populations includ-
ing the West African Mandenka and Yoruba, the Southern
African San, and South West Bantu. The relatively high fre-
quencies of the derived allele in Central Asian, Middle
Eastern, and North Africa seem likely to be due to gene flow
with European populations. Similarly, the presence of the
derived allele (albeit at low frequencies) in some sub-Saharan
African populations may be due to recent gene flow from
European and Central Asian populations. Alternatively, the
derived allele may have existed in the ancestral human pop-
ulation and was lost in the ancestors of modern East Asians
but retained in the ancestral European population. The al-
lele then rose to high frequency in Europeans following the
divergence of European and East Asian ancestral groups.

These results simultaneously and strongly suggest that
Europeans and East Asians have evolved lighter skin inde-
pendently and via distinct genetic mechanisms, as there is
an absence of any unusual pattern of diversity at SLC24A5,
MATP, and TYR in East Asians. These observations are
consistent with conclusions based on analyses of heterozy-
gosity levels surrounding the SLC24A5 functional poly-
orphism in the HapMap populations (Lamason et al.
2005), the results of a resequencing study of MATP (Soejima
et al. 2005), and a recent work examining variation in
SLC24A5 and MATP in populations representing Europe,
Africa, and Asia (Soejima and Koda 2006). Assuming that
the lighter pigmentation phenotype of both Europeans and
East Asians is due to the same selective pressure (decreased
UVR), we might expect to identify other loci having phe-
notypic effects as strong (or stronger) than these 3 loci that ex-
hibit patterns of reduced diversity and population-specific
FST in East Asian populations. Intriguingly, analyses of
HapMap data corroborate this suggestion in identifying 2
a priori pigmentation candidate genes, ADAM17 and ATRN,
that show evidence of strong, population-specific selection
in East Asians. These loci show extended blocks of signif-
icant values at all 3 test statistics computed for the HapMap
data (supplementary table 3, Supplementary Material on-
line) in East Asian populations. Although both of these
genes are associated with pigmentation variation in mice,
they also have other effects outside of the pigmentation sys-
tem, including the development of the central nervous sys-
tem and energy homeostasis (ATRN) (Lu et al. 1999; He
et al. 2001; Barsh et al. 2002) and development (ADAM17)
(Peschon et al. 1998). As such, further investigation will
be required to confirm the role of these genes in regulating
normal pigmentation variation as well as the potential se-
lective event that has shaped the observed patterns of ge-
netic variation.

The MC1R gene was the only locus examined in detail
that did not show any signal of potential positive selection.
Previous sequence-based studies have reached conflicting
conclusions about whether or not MC1R has been subject
to positive selection outside of Africa (Rana et al. 1999;
Harding et al. 2000; Makova et al. 2001). Although
MC1R’s association with red hair, fair skin, freckles, and
melanoma risk in European and European-derived popu-
lations primarily from the British Isles (Box et al. 1997; Smith
et al. 1998a; Schiöth et al. 1999; Flanagan et al. 2000;
Bastiaens et al. (2001) clearly demonstrates the important regional role that it plays in pigmentation, MC1R may have (with some exceptions [John et al. 2003; Nakayama et al. 2006]) little effect on variation outside of Europe (Myles et al. 2006). Consequently, no signal will be detected using our approaches. Although the 2 SNPs that we typed in MC1R are not strongly associated with the red hair and fair skinned phenotype for which MC1R is so well known (Sturm et al. 2003), both are polymorphic in global surveys of populations (Rana et al. 1999; Harding et al. 2000). In addition, the MC1R G92A SNP may have a “mild” effect on pigmentation phenotype (Motokawa et al. 2006). The 92A allele at this site is known to have a lower affinity for α-MSH than wild-type MC1R alleles (Xu et al. 1996), which suggests that it may contribute to normal variation in pigmentation. However, if positive directional selection has acted on MC1R, we would expect variation at linked sites to be affected. As such, even if we have not assayed the relevant functional SNP, we should still have observed some signal of selection, especially given the small size (~3 kb) of this gene.

Two recent papers have examined signals of selection in pigmentation candidate genes using publicly available data from the HapMap and Perlegen databases that contain genotype information from African (or African-American), European, and East Asian populations (Izagirre et al. 2006; Myles et al. 2006). Both studies examine $F_{ST}$ and measures of the extent of long-range haplotypes (LRHs) surrounding a number of candidate loci in these 3 populations. All 3 studies have reached similar conclusions for a subset of the genes examined. Both Izagirre et al. (2006) and Myles et al. (2006) identified signals of European-specific selection at SLC24A5. Similarly, both studies also identified signals of selection in MATP between Europeans and East Asians. However, Izagirre et al. (2006) did not detect a signal at MATP between Europeans and Africans. We agree with Myles et al. (2006) that this discrepancy may be due to the sampling strategy of Izagirre et al. (2006), in which allele frequency information was pooled across both Africans and African-Americans. At OCA2, Myles et al. (2006) reported $F_{ST}$ values that, although not statistically significant, were suggestive of European divergence at OCA2, similar to our $F_{ST}$ results. All 3 studies failed to observe a signal of selection at MC1R. Although all 3 studies agreed on the potential role of selection in the above-mentioned genes, at other loci, there is some disagreement between the 3 works. Neither Izagirre et al. (2006) nor Myles et al. (2006) observed significant $F_{ST}$ or LRH values at TYR. Although we did observe significant $F_{ST}$ values between Europeans and all other populations at this locus, we did not observe a gene-wide signal at TYR in our HapMap analyses, suggesting that this difference may be due in part to the populations and types of analyses used in each study. These differences may also explain why Izagirre et al. (2006) and Myles et al. (2006) failed to detect a signal of European-African differentiation at ASIP. Although we only examined 6 genes for significantly high $F_{ST}$ differences, we also examined an additional 28 pigmentation candidate loci for signals of selection using HapMap data alone. These results also show some agreement with Izagirre et al. (2006) and Myles et al. (2006), including a signal of East Asian differentiation at ADAM17 (Myles et al. 2006) and European differentiation at TYRP1. Finally, Myles et al. (2006) identified DCT as a candidate for influencing pigmentation in East Asian populations (but importantly, not in European populations). Although we did not include DCT in our $F_{ST}$ analyses, it did show significantly high LSLB in the HapMap East Asian sample.

Observed patterns of global skin pigmentation diversity and their correlation with environmental UV exposure suggest an adaptive response. Although we cannot rule out a role for sexual selection, our results support multiple genetic mechanisms for evolution of skin color. We provide evidence that at least 2 genes, ASIP and OCA2, probably play a shared role in shaping light and dark pigmentation across the globe. We have also firmly identified a further 3 genes (consistent with the results of previous studies: Lamason et al. 2005; Soejima et al. 2005), and potentially several more candidate loci, that have a significant effect in regional pigmentation phenotype. Our data strongly support independent genetic origins for the light skin phenotype in Europeans and East Asians arising after the divergence of modern European and East Asian populations.

**Supplementary Material**

Supplementary tables 1–3 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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**Literature Cited**


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