A Scan for Signatures of Positive Selection in Candidate Loci for Skin Pigmentation in Humans

Neskuts Izagirre,* Iker García,* Corina Junquera,† Concepción de la Rúa,* and Santos Alonso*

*Department Genetics, Physical Anthropology and Animal Physiology, Faculty of Science and Technology, University of the Basque Country, Leioa, Bizkaia, Spain and †Progenika Biopharma, S.A., Edificio 801A, Parque Tecnológico de Zamudio, Derio, Spain

Although the combination of pale skin and intense sun exposure results in an important health risk for the individual, it is less clear if at the population level this risk has possessed an evolutionary meaning. In this sense, a number of adaptive hypotheses have been put forward to explain the evolution of human skin pigmentation, such as photoprotection against sun-induced cancer, sexual selection, vitamin D synthesis or photoprotection of photolabile compounds, among others. It is expected that if skin pigmentation is adaptive, we might be able to see the signature of positive selection on some of the genes involved. In order to detect this signature, we analyze a battery of 81 candidate loci by means of phylogenetic and population genetic tests. Our results indicate that both light and dark skin may possess adaptive value. Of the main loci presenting this signature, TP53BP1 shows clear evidence of adaptive selection in Africans, whereas TYRP1 and SLC24A5 show evidence of adaptive selection in Caucasians. Although we cannot offer a mechanism that based on these genes explains the advantage of light skin, if TP53BP1, and perhaps RAD50, have truly conferred an adaptive value to the African population analyzed, photoprotection against sun-induced skin damage/cancer might be proposed as a mechanism that has driven the evolution of human skin pigmentation.

Introduction

The apportionment of human diversity in skin color is atypical. Although genetic markers indicate that the differences among major geographical groups represent only a 10% of the total human genetic diversity (Lewontin 1972; Barbujani et al. 1997), variation in skin reflectance among populations of the major geographical regions represents an 88% of the global diversity (Relethford 2002). As these differences might be adaptive, the pigmentation of the human skin represents a topic of special interest in human evolution (Diamond 2005). Moreover, given the evidence that intense sun exposure and pale skin might be important risk factors in skin cancer (Sturm 1998; Sturm et al. 2001; Agar and Young 2005 and references therein), the pigmentation of the skin also represents a topic of health concern.

However, although this health risk is evident, it is less clear that protection against skin cancer is the driving mechanism in the evolution of human pigmentation. Despite the appeal of this hypothesis, evidence supporting it has been indirect, and thus, a number of hypotheses coexist on the mechanisms by which pigmentation offers an adaptive value (Robins 1991; Quevedo and Holstein 1998; Aoki 2002; Jablonsky 2004). There is evidence of correlation between skin reflectance and environmental variables such as UV radiation (Jablonsky and Chaplin 2000; Chaplin 2004; Jablonsky 2004). It is expected that positive selection may lead to an increase in the values of this statistic when comparing populations under different environmental pressures. Similarly, the extended haplotype homozygosity (EHH) test (Sabeti et al. 2002) can detect the presence of adaptive mutations by analyzing long-range single-nucleotide polymorphism (SNP) haplotypes and identifying regions that show unusually high EHH and high population frequency.

More than 100 genes have been identified in the mouse in which mutations can affect pigmentation of the skin, hair, or eyes. Many of them have been cloned, and for all of these, a human orthologue has been identified (Bennet and Lamoreux 2003). Among these, MCIIR has been the locus most extensively investigated for polymorphisms and divergence (Valverde et al. 1995; Healy et al. 1999; Rana et al. 1999; Harding et al. 2000; Box et al. 2001; Mundy and Kelly 2003). Herein, we apply these 3 approaches to a battery of
81 loci. Overall, these loci are involved in a variety of physiological processes, including DNA damage response genes, immunity and hypo/hyperpigmentation-related genes (Stinchcombe et al. 2004), melanocyte migration, control of melanogenesis, hormone response genes (like γ-MSH and the sex hormones or vitamin D), or prostat glandin-coding genes (prostaglandins may mediate postinflammatory pigmentary changes through modulation of melanocyte dendricity and melanin synthesis) (Scott et al. 2004). Our results indicate that both light and dark skin may possess adaptive value in human populations.

Materials and Methods

The selection of loci involved in pigmentation included mainly, but not exclusively, the cloned mouse color genes listed on the Web page of the International Federation of Pigment Cell Societies (http://ftp.csc.med.umich.edu/micemut.htm). Another set of genes was selected from the literature on the pigmentation patterns of Drosophila. Other genes were chosen by their association to hormones or other molecules that have been hypothesized to have a role in pigmentation. The initial coding sequences of the human, mouse, and chimpanzee orthologues were downloaded from the University of California Santa Cruz (UCSC) genome browser (http://genome.ucsc.edu/) and/or the Ensembl genome server (http://www.ensembl.org/index.html). Occasionally, other species, including other primates or mammalian genomes, were used instead of or in addition to that of the mouse. In case of alternative transcripts, the isoform with the greater number of exons and/or that maximizing the global alignment was selected. Sequences were edited with BioEdit 5.0.9 (Hall 1999). Before phylogenetic analysis, both initiation and stop codons were trimmed. Occasionally, some segments that were difficult to align with confidence were removed from the alignment. Small alignments were carried out manually when necessary; occasionally, ClustalW was run to help obtain a global alignment. Alignments were always inspected by eye. In case of conflicting sequence data for a particular species, we conservatively favored the variant that maximized global sequence similarity. Loci that were too divergent to align confidently were dropped from the study. Finally, a set of 81 loci was selected for phylogenetic analysis. FASTA alignments are available as Supplementary Material 1 online. These loci include ADAM17, ADAMTS20, AP3D1, AP3M2, AR, ASIP, ASMT, ATRN, BLOC1S3, BRCA1, CHS1 (LYST), CNO, CYP24A1, CYP27B1, D6C, DDC, EDA, EDN3, EDNRB, ERCC2, ESRI, ESRR, GGT1, GPR143, HP51, HP52 (AP3B1), HP53, HP55, HP56, HP57 (DTNB1), IKBKG, KIT, KITLG, MAGMAS, MAMAL1, MATP (AIM1), MC1R, MDM2, MGRN1, MITF, MLANA, MLPH, MTRN1A, MTRN1B, MUTED, MYOSA, MYO7A, OCA2 (P locus), OST1, PAR2, PAX3, PER1, PGR, PKCB, PLDN, POMC, PTGER1, PTGER2, PTGER3, PTGER, PTGIR, RAB27A, RabBGTA, RAD50, RXRA, SILV (PM17), SLC24A5, SOX10, TBX2, TH, TP53, TP53BP1, TTYR1, TYP1, TYP2 (DCT), VDR, VDRIP, VPS18, VPS33A, YARS, and ZNFN1A1.

Phylogenetic analysis was conducted by means of the PAML package (Yang 1997). Thus, the maximum likelihood $d_\omega/d_s$ ratio ($\omega$) test was performed using codeml. For each locus, 3 pairs of models, representing the null versus the test hypothesis, were evaluated by the log-likelihood ratio test. First, we evaluated the one-ratio branch model (assuming one $\omega$ for all lineages, model = 0, NSsites = 0) versus the free-ratio branch model (in which $\omega$ is free to vary for each branch, model = 1, NSsites = 0) (Yang 1998). A second test evaluated the sites model M1 (model = 0, NSsites = 1) versus the branch-sites model A (model = 2, NSsites = 2), an extension of model M1. M1 assumes 2 categories of sites, the first includes neutral sites, for which $\omega_1 = 1$, and the second category includes conserved sites with $\omega_2 = 0$; this is called the “neutral model.” In branch-sites models, $\omega$ varies among branches and codons, with a total of 4 site classes in the sequence: there are 2 site classes along the background lineages with ratios $\omega_2$ or $\omega_1$ (sites with $\omega_0$, a small $\omega$, are highly conserved; sites with $\omega_1$, with $\omega$ near or smaller than 1, are neutral or weakly constrained sites), but along the lineage of interest, the foreground lineage, a certain event caused some sites to come under positive selection and have a $\omega$ ratio $\omega_2 > 1$. In model A, $\omega_0$ and $\omega_1$ are fixed to 0 and 1, respectively. Third, we evaluated the sites model M3 (model = 0, NSsites = 3) versus the branch-sites model B (model = 2, NSsites = 3). M3 is an unconstrained discrete model that accommodates heterogeneous $\omega$ ratios among sites. Model B is similar to A, but $\omega_0$ and $\omega_1$ are estimated from the data as free parameters. Model B is an extension of the sites model M3 (Nielsen 1998; Yang et al. 2000; Yang and Nielsen 2002). Finally, as the above tests have been recently considered as excessively “liberal” (Zhang 2004), those loci that showed a significant result with the above tests were also subject to the improved “test 2”: in this test, the null hypothesis is the branch-site model A but with $\omega_2$ fixed to 1; its alternative model has $\omega_2 \geq 1$ constrained (Zhang et al. 2005). Results for the 81 loci are available as Supplementary Material 2 online.

For the $F_{ST}$ comparisons, population SNP frequencies from populations from 3 main groups, Caucasians, Africans, and East Asians, were selected using online resources like the Gene Genotype Report link in the UCSC genome browser (http://genome.ucsc.edu/) and SNP Browser version 2 (Appelera Corporation 2004) (http://marketing.appliedbiosystems.com/mk/get/snph_landingsource=f_r_E_RD_www_allsnps_com_snspbrowser). To avoid bias, more than 1 source of frequency data for the 3 main groups were used when possible and merged. It is expected that if different sources provide frequency information for the same SNPs, then the different strategies for SNP detection should not be creating major artifacts in this regard. These sources were mainly 1) the Perlegen data (Hinds et al. 2005), from which frequency data were collected for about 77% of the SNPs and which includes 71 individuals of European American, African-American, or Han Chinese ancestry; 2) the Cold Spring Harbor Laboratory (CSHL)-HapMap data, from which frequency data were collected for about 67% of the SNPs and which included 120 Yoruba in Ibadan, Nigeria; 90 Japanese in Tokyo, Japan; 90 Chinese in Beijing, China, and 120 Utah Residents with Ancestry from northern and Western Europe (The International HapMap Consortium 2003); and 3) the data available through SNPBrowser (Appelera
Corporation 2004) (including 45 African-American, 45 Caucasian individuals from the Coriell Institute Repository, 45 Chinese, and 45 Japanese) which was used for less than 10% of the SNPs. Chinese and Japanese data were subsequently merged. Only alleles with minor allele frequency greater than or equal to 0.1, at least in one population, were considered. However, a minor allele frequency of 0.2 or more was preferred if more than one SNP were available in close vicinity (ca. ±/– 2 kb). Only SNPs under Hardy–Weinberg equilibrium (HWE) were considered. For some of the SNPs that were not in HWE according to the classical χ² test due to the low absolute frequency of some genotype classes, an exact test was performed. For SNPs on the X chromosome, HWE was tested using only the data from the female individuals in the samples. Unbiased estimates of FST were calculated as described (Weir and Cockerham 1984; Akey et al. 2002). Thus,

\[ F_{ST} = \frac{\text{MSP} - \text{MSG}}{\text{MSP} + (n_i - 1)\text{MSG}}, \]

where MSP denotes the observed mean square errors for loci between populations

\[ \text{MSP} = \frac{1}{s-1} \sum_{i=1}^{s} n_i (p_{Ai} - \bar{p}_A)^2, \]

s being the number of subpopulations, \( p_{Ai} \) the frequency of allele \( A \) in population \( i \), and

\[ \bar{p}_A = \frac{\sum_i n_i p_{Ai}}{\sum_i n_i} \quad \text{and} \quad n_i = \frac{\sum_j n_{ij}}{s-1} \sum_i n_i - \sum_{i} n_{ij}, \]

\( n_i \) denoting the sample size in subpopulation \( i \). Finally, MSG denotes the observed mean square error for loci within populations

\[ \text{MSG} = \frac{1}{s-1} \sum_{i=1}^{s} n_i p_{Ai} (1 - p_{Ai}). \]

\( F_{ST} \) results for the 81 loci are available as Supplementary Material 3 online. As pointed out in Akey et al. (2002), genotyping errors are unlikely to produce false positive results as, on the one hand, the final allele frequencies employed are the result of lumping together 2 different data sets for each geographical group (or 3 in the case of East Asians) in a majority of the cases and, on the other, genotyping errors per se are more likely to decrease \( F_{ST} \) values.

To obtain a neutral distribution of the \( F_{ST} \) statistic, we selected 43 regions distributed across the autosomal genome that belong to broader regions of low gene density and which are separated at least 150 kb from the closest exon. Each of these regions spans an average of 1.96 Mb and in total make 84.3 Mb. For each region, we downloaded the SNP frequency information available from the HapMap browser (data Rel #20/phase II on National Centre for Biotechnology Information BiB35 assembly, dbSNP b125) for the 3 major populations (Caucasians: 153,339 SNPs, Yorubans: 123,798 SNPs, and Chinese: 33,190 SNPs). We further filtered the number of SNPs to include only those SNPs 1) that were separated at least 100 kb from each other, 2) that have been genotyped in all 3 populations, and 3) for which at least one of the 3 major populations has a minor allele frequency higher than 0.1. A final list of 546 SNPs satisfied these criteria. Therefore, we have used the upper 95% limits of these observed “neutral” distributions as the cutoff points to declare departure from neutrality. The 95% upper limits were Caucasians–Chinese: 0.33, Caucasians–Yorubans: 0.47, and Chinese–Yorubans: 0.45 (fig. 1).

Regarding the observed EHH distributions, these were obtained by following the method developed in Sabeti et al. (2002). Basically, whole chromosome SNP information for 3 populations, Africans, Caucasians, and Chinese was downloaded from the HapMap Web page (http://www.hapmap.org). In general, for each gene of interest, we selected a region that, centered on the gene as much as possible, extended approximately 200 kb on each direction. For this region, we looked for a central core region of 3–5 SNPs long that followed the infinite sites model in the global population sample. This core region defined core haplotypes for which their frequency was registered. The core haplotype with the highest frequency for each population was analyzed for their EHH. In approximately 10% of the loci, the second most frequent core haplotype in a given population was also analyzed, particularly, if this core haplotype was present with high frequency in non-Africans and was different from the most frequent core haplotype in Africans. For this analysis, haplotype homozygosity was scored starting with the core haplotypes and increasing their length in one SNP each time. In order to test the significance of the extended homozygosity distribution, we obtained the neutral distributions by running simulations using RR Hudson’s ms program (http://home.uchicago.edu/~rhudson1/source.html) under the following demographic scenario: we assumed an Out-of-Africa model in which 3 demographic splits take place. One, 2,500 generations ago, reflecting the dispersals of modern humans through Europe; a second, 3,000 generations ago, reflecting the colonization of Asia...
and a third one, 5,000 generations ago reflecting the Out-of-Africa expansion. \( N_e \) was assumed to be 10,000, and \( \theta = 4N_e \mu \) was 160 for a DNA segment of 200-kb long. For the Caucasian and East Asian populations, an exponential population growth parameter \( \alpha = 2N_e \) was set to 40. It is expected that doing so, the effect of population growth can be discarded when explaining possible departures from neutrality. In order to be conservative, no migration between demes was considered. Recombination was included in the simulations by taking the Decode sex averaged recombination rates displayed in the UCSC genome Web browser for each gene (http://genome.ucsc.edu). Parameters were proportionally reduced according to the frequency of the haplotype being simulated by using the \(-n\) flag. Initially, for each of the approximately 200-kb-long-regions, 2,000 simulations were run, and a \( P \) value of 0.05 was used as the significance cutoff point. From each simulation, a Perl script randomly chose a number of SNPs identical to that shown by the HapMap Web page for each region under study. Thus, from the whole set of simulations, we obtained the upper 95% EHH values for each nonoverlapping window of 1-kb long. If the observed EHH values were higher than the 95% upper EHH values obtained in the corresponding simulated distribution, we considered that as evidence of positive selection. In order to correct for multiple testing, we used a \( P \) value of 0.005 which rendered in this case an approximate, conservative, false discovery rate (FDR) of 4%, estimated from the approach of Benjamini and Hochberg (1995) to control FDR at a level \( \alpha \). This approach ranks the initial \( P \) values in ascending order \( P(1) \leq P(2) \leq \cdots \leq P(m) \), \( m \) being the number of tests and specifies \( P(i) \leq \alpha i/m \) as the point below which there is no rejection at a FDR \( \alpha \). To obtain the multiple-test–corrected upper confidence limits of the EHH distribution for each window of 1-kb long, 5,000 simulations were run.

**Results**

Validation of the Phylogenetic and \( F_{ST} \) Methods

Firstly, we tested if the proposed phylogenetic approach may be useful to detect adaptive selection, even when selection has acted only in one geographical population group. For that, we subjected a set of 10 genes from those described in Akey et al. (2004) to the phylogenetic test described above (see Materials and Methods). This set of genes had been analyzed previously for evidence of selection with pairwise Tajima’s \( D \), Fu and Li’s \( D^* \), Fu and Li’s \( F^* \), and Fay and Wu’s \( H^* \) tests in African-Americans and European Americans. Five of these loci (DCN, EPHB6, KEL, TRPV5, and TRPV6) were identified in Akey et al. (2004) as “positively selected” in European Americans (but not in African-Americans), and for the other 5 (IL1B, F2RL1, TNF, FGB, and THBD), no robust evidence of selection was found in either group. Thus, 3 loci (EPHB6, TRPV5, and TRPV6) also showed significant evidence for positive selection in at least one of the phylogenetic tests described. Of these 3 loci, TRPV6 was also significant for test 2 (–2LR = 51.1; df = 1). DCN and KEL showed \( \omega > 2 \) for some nucleotide positions in all 3 species. For the rest of the loci, the phylogenetic tests provided evidence of predominant purifying selection or evidence of neutrality (with some sites with \( \omega \) values between 1 and 1.2).

Secondly, to test the \( F_{ST} \) method, all these 10 loci were also subject to \( F_{ST} \) analysis (using the same criteria and sources of data as described in Materials and Methods). In this case, the group of previously declared positively selected loci in Caucasians (DCN, EPHB6, KEL, TRPV5, and TRPV6) showed, as a whole, average pairwise \( F_{ST} \) values of 0.44 for Caucasians versus Africans, 0.01 for East Asians versus Caucasians, and 0.36 for East Asians versus Africans. EPHB6, KEL, TRPV5, and TRPV6 showed SNPs with significant values for Caucasians versus Africans at a FDR (Benjamini and Hochberg 1995) of approximately 17%. In contrast, for the set of loci with no robust evidence of selection, average \( F_{ST} \) values of 0.04, 0.05, and 0.14 were found, and no particular SNP showed significant \( F_{ST} \) values in any pairwise population comparison.

Phylogenetic Results

A total of 81 loci related, in the broad sense, to the pigmentation phenotype were scanned for phylogenetic evidence of selection. The phylogenetic tests indicate that initially only 4 genes showed a significant signal of differential positive selection in the human lineage. These loci are BLOC1S3, BRCA1, MYO7A, and PGR. Of these, BRCA1 and PGR had already been identified as under positive selection in humans (Yang and Nielsen 2002; Nielsen et al. 2005, respectively). However, after multiple test correction, only MYO7A and PGR remained significant (at a FDR value of ca. 4%). Twenty-five loci showed nonsignificant evidence of positive selection but with likelihood ratios supporting it, at least in one of the 3 phylogenetic tests: ADAMTS20, AR, ASIP, CYP24A1, DC6, DDC, ESRI2, GPR143, HPS2, HPS3, IKBKG, LYST, MCRN1, MDM2, PAR2, PER1, PTGER1, PTGFR, PTGIR, RAB27A, SOX10, TP53BP1, TYR1P, TYRP2, and OCA2. Fifteen loci, ASMT, CYP27B1, EDN3, HPS1, KITLG, MAGMAS, MITF, MLANA, OSTM1, PTGER2, RAD50, TBX2, TH, TYR, and VDRIP show some evidence of positive selection but not exclusive to humans. Twenty-eight genes showed evidence of being or having been under a regime of negative
selection: ADAM17, AP3M2, ATRN, CNO, DTNBP1, EDA, EDNRB, ERCC2, GGT1, HP56, KIT, MC1R, MTNR1B, MUTED, MYO5A, PAX3, PKCβ, PLDM, PTGER3, POMC, RABGGTA, RXRA, TP53, VDR, VPS18, VPS3A, YARS, and ZNFN1A1. Eight additional genes: ESR1, HP55, MAL1, MAP2K5, MTNR1A, SILV, and SLC24A5 were classified as “neutral” as a proportion of sites showed α values about 1. AP3D1 was left as unclassified.

Population Genetic Results

F_{ST} Values

We finally decided not to consider those loci on the X chromosome (GPR143 with 2 SNPs, AF with 7 SNPs, EDA with 18 SNPs, and IKBKG, with no SNPs available) due to the small sample size remaining after removing the male individuals. In addition, BLOC1S3 could not be evaluated for F_{ST} as no SNPs satisfying our criteria could be retrieved. Thus, a total of 338 SNPs belonging to 76 autosomal loci from those listed above were compared for their frequencies in the 3 populational groups (frequency data on Chinese and Japanese were pooled). SNPs within the same gene were separated from each other. 15 kb on average approximately, which given the average sizes of linkage disequilibrium blocks in human populations (Hinds et al. 2005) should be enough to detect the possible effects of selection on most nucleotides across each gene even if these effects are indirect, that is, hitchhiking. For these loci, the global observed F_{ST} distribution showed parameters similar to those described using a more comprehensive set of loci by Akany et al. (2002). Thus, 17.5% of the F_{ST} values among the 3 populations were equal to 0, a 7.7% of the F_{ST} values were equal to or greater than 0.4, and the average F_{ST} was 0.135, whereas Akany et al. (2002) analysis yielded values of 10.8%, 4%, and 0.122, respectively. However, the observed F_{ST} distribution obtained from 81 candidate loci was different from the neutral one (Kolmogorov-Smirnov D value 0.21, P = 0) and from the distribution obtained by Akany et al. (2002) (Kolmogorov–Smirnov D value 0.12, P = 0.002). Summarized pairwise population comparisons of F_{ST} distributions are shown in figure 1. Of the total 338 SNPs, 61 (belonging to 29 loci; Supplementary Material 3, Supplementary Material online) showed at least one significant pairwise F_{ST} value (equal to or higher than the 95% upper limits of the observed neutral distribution). 61 SNPs were distributed across all of the above phylogenetic categories of loci.

Aiming at identifying F_{ST} differences that parallel broad phenotypic differences in skin pigmentation, we initially decided to consider an SNP as “pigmentation relevant” when the following criteria were met: 1) we applied a multiple test correction (with a FDR of 35%); 2) F_{ST} values had to be “consistent,” that is, F_{ST} values had to follow a pattern whereby when the Caucasian (East Asian) population presented a multiple-test–corrected significant F_{ST} value with the Yoruban population, the East Asian (Caucasian) population had also to show an F_{ST} value with the Yoruban population higher than the pairwise F_{ST} value between Caucasians and East Asians; 3) the pairwise comparison involving both non-African populations had to show a nonsignificant F_{ST} value.

The rationale of using these criteria, whereby our East Asians have to follow the same trend as Caucasians, is based on the phenotypic measures of skin pigmentation. The average skin reflectance (specifically for melanin, filter 609) varies little between Caucasians and the East Asians. Thus, the British population has an average skin reflectance of roughly 66–69%, whereas the Japanese population, for instance, has an average reflectance of approximately 51–60% (Robins 1991). However, these differences are more apparent when considering the African populations. Although for Africans, reflectance values can range from as low as ~18% to as high as 50% or more, for Nigerian Yorubans (the African population in the CSCHL-HapMap panel), population average skin reflectance ranges between 24% and 26% (Robins 1991). After applying the criteria described above, only TP53 and TP53BP1 showed one pigmentation relevant SNP each (rs2287499 and rs2467737, respectively).

However, Lamason et al. (2005) have recently shown that population-specific differences in skin pigmentation genes may exist. In this sense, SLC24A5 showed multiple-test–corrected, significant differences between Caucasians and Africans only, and 9 loci (ADAMT20, ADAMS20, ESRR2, LYST, MATP, OCA2, PTGER1, TYRP1, and TYRP2) showed multiple-test–corrected, significant differences between Caucasians and East Asians only.

EHH Test

This test was applied to a selection of 32 loci from those analyzed before. The list included all 4 loci (BLOC1S3, BRCA1, MYO7A, and PGR) showing phylogenetic evidence of positive selection at least with one of the tests described above, in addition to those 28 loci not already counted in and that showed at least one significant, multiple-test–uncorrected, pairwise F_{ST} value: ADAM17, ADAMS20, ATRN, EDN3, ESRR2, KIT, KITLG, LYST, MATP, MDM2, MIF, MLPH, MUTED, OCA2, PAR2, PAX3, PKCβ, PTGER1, RAB27A, RAD50, SLC24A5, TH, TP53, TP53BP1, TYR, TYRP1, TYRP2 (DCT), and VDRIP. This short list allowed us to reach an equilibrium between unrestricted inclusiveness and exclusion of those with less chance to produce positive results in order to reduce the number of analyses.

Out of these 32 loci, 9 (ADAMS20, ATRN, BRCA1, MLPH, LYST, PGR, TP53BP1, RAD50, and VDRIP) showed one haplotype with a significant signature of recent positive selection in a region within or around the locus. However, after applying a correction for multiple tests, only 5 loci remained significant (at a FDR of ca. 4%). Three loci (LYST, TP53BP1, and RAD50) showed this signature only in the Yoruban population (fig. 2) and 2 loci (TYRP1 and SLC24A5) only in Caucasians (fig. 3).

Discussion

We have made use of the available public genomic information to scan for signatures of selection in candidate loci for skin pigmentation by conducting 3 complementary tests. The phylogenetic tests employed search for evidence
of selection by looking exclusively at the coding sequence of the loci, whereas the other 2 tests ($F_{ST}$ and EHH) use the information on coding and (mostly) noncoding SNPs distributed along the locus of interest and surrounding regions. Due to SNP ascertainment bias, other tests for selection based on the number of singleton haplotypes are not applicable to this kind of data (Voight et al. 2006). It might be expected that the phylogenetic test would provide us with evidence of selection on the human lineage as a whole, whereas the other 2 tests are more likely to highlight geographically specific or population-specific processes. Positive selection at the phylogenetic level may be difficult to detect because it often operates episodically on a few amino acid sites, and the signal may be masked by negative selection. (Zhang et al. 2005). In addition, the reduced number of species used in this work in many of the comparisons, the short length of the coding sequence of some of the loci and the conservative approach used in the alignment might lead to lack of power to detect positive selection with statistical significance. However, we have shown that these tests can detect population-specific signatures of selection (see TRPV6 above, see also Akey et al. 2004 for additional discussion on this aspect). Despite these caveats, some of the phylogenetic tests identified 2 loci, MYO7A and PGR, as under positive selection. PGR was included in the list of candidate loci based on suggestions that both progesterone and estrogen may have a strong impact on the pigmentation of sexual skin (Robins 1991). MYO7A was included in our list of candidate genes as it has been reported to regulate melanosome transport, at least in the retinal pigmented epithelium (Gibbs et al. 2004). However, the functions of these loci are not restricted to the melanocyte. PGR is involved in the reproductive process, and MYO7A also functions as a lysosome-associated molecular motor in sensory cells (Soni et al. 2005). Therefore, whether these 2 loci have hold an adaptive value because of their role in the evolution

**Fig. 2.**—Decay of homozygosity in the EHH test for RAD50, TP53BP1, and LYST in Yorubans. The plots show the observed extent of homozygosity and confidence intervals (CIs) in the 3 loci significant for the EHH test in Yorubans: (a) RAD50, (b) TP53BP1, and (c) LYST. The small gray dots represent the 95% upper limits of the EHH distribution obtained by simulations. The discontinuous line shows the 99.5% upper limits (representing an FDR value of ca. 4%). The bigger black dots represent the observed EHH values. Above each graph, the approximate localization of the loci under study and their surrounding neighbors is indicated by arrows pointing in the sense of transcription.
of skin pigmentation or in other processes cannot be inferred from this analysis.

Of the other 2 tests, \( F_{ST} \) is expected to have the lowest power to detect departures from neutrality as this test simply utilizes SNP frequency differences between populations, and frequencies may show a wide variance given the stochastic nature of neutral evolution. We cannot exclude either the possibility that other mechanisms like epistatic interaction may pass undiscovered by this single locus approach. This effect may be more pronounced if pigmentation is also the result of multiple minor QTLs. The EHH test has been reported to lead to greater power to detect selection by making use of more informative parameters, like the decay of homozygosity (Sabeti et al. 2002). With this in mind, 5 loci have been initially identified as positively selected. Three of them (LYST, TP53BP1, and RAD50) show this evidence for positive selection in the African population only (fig. 2). The other 2 loci (TYRP1 and SLC24A5) are positive in Caucasians only.

Of these, LYST belongs to the group of immunity and hypo/hyperpigmentation-related genes. There are a number of diseases characterized by pigmentation defects and simultaneous immune dysfunction (Stinchcombe et al. 2004). Some of them, like Chediak–Higashi syndrome (CHS) in humans (OMIM 606897), caused by mutations in \( LYST \) are characterized by the presence of enlarged dysfunctional secretory lysosomes, dysfunction restricted to immune cells and melanocytes. In melanocytes, this dysfunction affects the melanosomes (melanocyte lysosomal-related organelles that release melanin to neighbor keratinocytes), which results in a marked associated hypo-pigmentation. Thus, the relationship between mutations in \( LYST \) and melanosomal size immediately suggests a possible role for variants of this group of genes in normal variation in skin pigmentation as melanosomal size is a trait directly related to the degree of skin pigmentation. Thus, in darkly pigmented individuals, the basal keratinocytes tend to have more abundant and larger melanosomes than Caucasians or Asians. It is interesting to note that of the 2 described alternative transcripts of \( LYST \), only the longer isoform shows severe reduction in abundance in CHS patients (Barbosa et al. 1997). It is precisely on the specific sequence corresponding to this isoform (the 3‘ end) that the signal of positive selection is observed (fig. 2). In this sense, we can speculate that the patterns of diversity in \( LYST \) favor darker skin in Africans, but however, we cannot discard that this effect might be due to the role of this gene in the immune response.

\( TP53BP1 \) (tp53-binding protein 1) is a putative early DNA damage sensor which has been labeled as a central transducer of the DNA damage signal to other tumor suppressor proteins and which is likely to play an important role in the maintenance of genomic stability and prevention of cancer (Wang et al. 2002). It has been reported that the principal mediators of the cellular transcriptional response to UV radiation are unrepaired cyclobutane pyrimidine dimers (CPDs), rather than other DNA lesions or other damaged macromolecules (Garinis et al. 2005). In this regard, CPDs provoke accumulation of \( TP53BP1 \) and their continuing presence appears to be the primary cause of the vast majority of (semi) acute responses in the UV-exposed skin, like sunburn, apoptosis, or skin cancer (Jans et al. 2005). The most prominent pathway induced by CPDs is that associated with DNA double-strand break signaling and repair. Interestingly, \( RAD50 \) is also involved in DNA double-strand break repair. The product of this gene forms a DNA-binding complex with MRE11 and NBS1 named the MRN complex, which acts at the very early steps of DNA repair (Valerie and Povirk 2003). However, it is not clear if the signal for positive selection deduced from the EHH graph (fig. 2) and \( F_{ST} \) values corresponds exclusively to this locus or to the effect of the

![fig. 3.—Decay of homozygosity in the EHH test for \( TYRP1 \) and \( SLC24A5 \) in Caucasians. The plots show the observed extent of homozygosity and CIs in the 2 loci significant for the EHH test in Caucasians: (a) \( TYRP1 \) and (b) \( SLC24A5 \). Interpretation as indicated in figure 2.](https://academic.oup.com/mbe/article-abstract/23/9/1697/1014248)
close neighbor locus IL-13. Other interleukins have been linked to melanogenesis. Thus, IL-1β has been shown to up-regulate the synthesis and release of ET-1 by keratocytes and α-MSH by human keratocytes and melanocytes, factors that stimulate cultured human melanocyte proliferation and melanogenesis (Abdel-Malek and Kadekaro 2006). Instead, IL-13 might possess an anti-inflammatory effect because treatment with IL-13 produced dose-dependent attenuation of UVB-induced hyperalgesia in mice (Saade et al. 2000). Alternatively, it might be the case that the signal for selection on RAD50 derives from the locus control region activity of the 3′ region of RAD50, which has been shown to influence the transcription of the pleiotropic cytokines IL-4 and IL-13 (Lee and Rao 2004).

These observations suggest that photoprotection against UV damage might have held an evolutionary value. It may be claimed that skin cancer, for instance, may cause little fitness reduction as it tends to happen after reaching reproductive age. However, in Nigeria and Tanzania, the proportion of albinos in the age range of 31–60 years has been shown to be 6%, a figure lower than that for non-albinos (20%). This observation may be linked to the fact that these albinos exhibit cancer or malignant lesions by the age of 20 (Robins 1991). Apart from cancer, sunburn has been pointed out another acutely disabling effect of sun overexposure, particularly by its interference with sweating (Robins 1991).

Our approach focused initially on those loci showing differences between populations of light skin and dark skin. However, other loci involved in the pigmentation phenotype may show more population-specific patterns of diversity. For instance, the “derived” state of SNP rs1426654 in SLC24A5, a putative potassium-dependent sodium/calcium exchanger gene, shows an allele frequency of 98.7–100% in several European American populations and has been claimed to have been the target of selection. However, in African and East Asian populations, it is the “ancestral” allele that showed frequencies between 93% and 100%. Besides, the role of this locus in pigmentation, at least in the zebrafish, has been confirmed experimentally (Lamason et al. 2005). We have confirmed that SLC24A5 may be under positive selection in Caucasians (fig. 3).

We also have observed in this work a similar pattern of EHH distribution supporting positive selection for TYRP1 in Caucasians only (fig. 3). TYRP1 is an enzyme that takes part in the eumelanin pathway. Its main function may be to regulate melanosome maturation, stabilize and maintain tyrosinase protein levels, suppress tyrosinase-mediated cell death protecting against the cytotoxicity of melanin intermediates, or act as a chaperone to tyrosinase (Kobayashi et al. 1998; Rad et al. 2004; Hearing 2005). Mutations in TYRP1 cause OCA3 (OMIM 203290), a form of oculocutaneous albinism characterized by normal amounts of tyrosinase but that shows a reduction in catalytic activity of 70% (Sturm et al. 1998). This role would agree with the observation that tyrosinase levels are similar among the main geographical human groups, but however, black skin have up to 10 times more tyrosinase activity than white skin (Iozumi et al. 1993). TYRP1 has been claimed to play a role in mediating ethnic differences in melanogenesis and constitutive pigmentation in vivo (Alaluf et al. 2003), and interestingly, a link between TYR, TYRP1, and UV irradiation by means of p53 has also been proposed (Nylander et al. 2000).

As the role for SLC24A5 in pigmentation has been functionally confirmed (Lamason et al. 2005) and as TYRP1 functions exclusively in melanogenesis, this indicates that light skin is not simply the result of loss of environmental pressure but that light skin likely holds an adaptive value too. In this sense, when a selected set of 8 SNPs, one per locus, from the DNA damage response group of loci (TP53, TP53BP1, MDM2, RAD50, and BRCA1) in addition to LYST, TYRP1, and SLC24A5 were chosen to run Structure v2.0 (Pritchard et al. 2000), the information contained in this set of SNPs seems to reflect overall patterns of phenotypic pigmentation (fig. 4).

The mechanisms by which light skin confers a selective advantage cannot be inferred with these data alone. However, the relevance of mechanisms like vitamin D synthesis, which have been claimed to have a role in the evolution of light pigmentation (Robins 1991 and references therein), could be minimized as neither of the representative loci of this pathway (Omdahl et al. 2002) that have been analyzed, VDR (Vitamin D receptor) and VDRIP (Vitamin D3 receptor-interacting protein), CYP27B1 (P450C1), CYP24A1 (P450C24), and RXRA (Retinoid X receptor), showed clear evidence of positive selection.

Thus, although human skin pigmentation may be the result of a complex interaction of environment and genes...
and of epistatic interactions among genes, many of which may have passed undetected, the present work highlights the likely adaptive value of both light and dark skin, being skin/DNA damage response a possible driving mechanism for the evolution of dark skin pigmentation.

**Supplementary Material**

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

**Web Site References**


UCSC genomes browser: http://genome.ucsc.edu/.


Structure v2.0: http://pritch.bsd.uchicago.edu/software/structure2_1.html.

ms program: http://home.uchicago.edu/~rhudson1/source.html.

**Acknowledgments**

We thank 2 anonymous reviewers, whose comments helped improve the content of the manuscript. S.A. is a Ramón y Cajal Fellow (Spanish Ministerio de Educación y Ciencia). I.G. is a PhD student granted by the Basque Government. Work funded by grants UE03/A02 and 9/UPV 00154.310-14495/2002 from the University of the Basque Country to C.R. and Bizkaitek 2003–2004 from the Diputación Foral de Bizkaia to S.A.

**Literature Cited**


Manolo Gouy, Associate Editor

Accepted June 1, 2006