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Human Pigmentation Variation: Evolution, Genetic Basis, and Implications for Public Health

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ABSTRACT Pigmentation, which is primarily determined by the amount, the type, and the distribution of melanin, shows a remarkable diversity in human populations, and in this sense, it is an atypical trait. Numerous genetic studies have indicated that the average proportion of genetic variation due to differences among major continental groups is just 10–15% of the total genetic variation. In contrast, skin pigmentation shows large differences among continental populations. The reasons for this discrepancy can be traced back primarily to the strong influence of natural selection, which has shaped the distribution of pigmentation according to a latitudinal gradient. Research during the last 5 years has substantially increased our understanding of the genes involved in normal pigmentation variation in human populations. At least six genes have been identified using genotype/phenotype association studies and/or direct functional assays, and there is evidence indicating that several additional genes may be playing a role in skin, hair, and iris pigmentation. The information that is emerging from recent studies points to a complex picture where positive selection has been acting at different genomic locations, and for some genes only in certain population groups. There are several reasons why elucidating the genetics and evolutionary history of pigmentation is important. 1) Pigmentation is a trait that should be used as an example of how misleading simplistic interpretations of human variation can be. It is erroneous to extrapolate the patterns of variation observed in superficial traits such as pigmentation to the rest of the genome. It is similarly misleading to suggest, based on the "average" genomic picture, that variation among human populations is irrelevant. The study of the genes underlying human pigmentation diversity brings to the forefront the mosaic nature of human genetic variation: our genome is composed of a myriad of segments with different patterns of variation and evolutionary histories. 2) Pigmentation can be very useful to understand the genetic architecture of complex traits. The pigmentation of unexposed areas of the skin (constitutive pigmentation) is relatively unaffected by environmental influences during an individual's lifetime when compared with other complex traits such as diabetes or blood pressure, and this provides a unique opportunity to study gene–gene interactions without the effect of environmental confounders. 3) Pigmentation is of relevance from a public health perspective, because of its critical role in photoprotection and vitamin D synthesis. Fair-skinned individuals are at higher risk of several types of skin cancer, particularly in regions with high UVR incidence, and dark-skinned individuals living in high latitude regions are at higher risk for diseases caused by deficient or insufficient vitamin D levels. Yrbk Phys Anthropol 50:85–105, 2007.

INTRODUCTION

Pigmentation is one of the most variable phenotypes in humans. The color of skin, hair, and eyes is primarily determined by melanin, a generic term used to describe a complex group of biopolymers synthesized by specialized cells known as melanocytes. The available evidence strongly suggests that natural selection is responsible for the observed variation in this trait, but the ultimate evolutionary factors have not been fully elucidated. There have been impressive advances in our understanding of the pigmentary system, mainly driven by studies in animal models, and important breakthroughs have also been made through studying pigmentation disorders in humans. In contrast, we are just beginning to understand the genetic basis of normal pigmentation variation in our species. In this article, I review the major evolutionary hypotheses that have been put forward to explain the distribution of pigmentation and the current state of our knowledge about the genes involved in the variation of skin, hair, and eye pigmentation within and among human populations. Finally, I devote the last section of this review to the important public health implications derived from the unique evolutionary history and geographic distribution of skin pigmentation.

A REVIEW OF PIGMENTATION BIOLOGY

Melanin is the main pigment of our skin, hair, and eyes, and other chromophores, such as hemoglobin, play a minor role in skin pigmentation. Melanin is not a single compound. Rather, it is a mixture of biopolymers synthesized in melanocytes located in the basal layer of the epidermis, the hair bulb, and the iris. Within the melanocytes, melanin production takes place in the melanosomes, lysosome-like organelles where melanin granules are synthesized using the aminoacid tyrosine as the major substrate. Tyrosinase (TYR) is the key enzyme in melanogenesis and mediates the first steps in melanin synthesis, which involve the hydroxylation of tyrosine to dopa and the subsequent oxidation to dopaquinone. Dopaquinone is a key intermediate compound that undergoes

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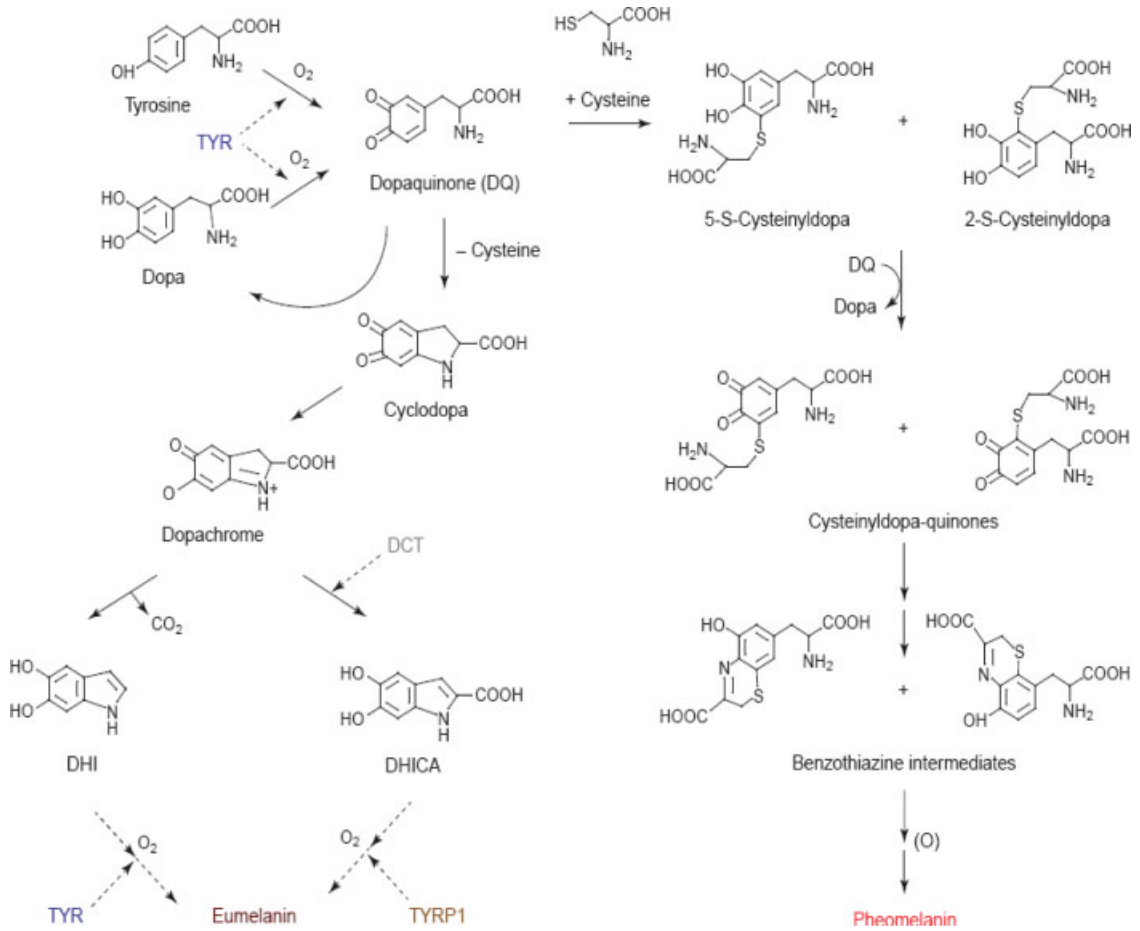

Fig. 1. Major steps in melanin synthesis. Reprinted from Sturm and Frudakis, 2004. Eye colour: portals into pigmentation genes and ancestry. *Trends in Genetics*. 20:327–332, with permission from Elsevier. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

further modification in two alternative pathways. In the absence of the amino acid cysteine, dopaquinone eventually gives rise to the dark brown/black, insoluble DHI (5,6-dihydroxyindole)-eumelanin and to the lighter, alkali-soluble, DHICA (5,6-dihydroxyindole-2-carboxylic acid)-eumelanin, while in the presence of cysteine, dopaquinone gives rise to the alkali-soluble red-yellow pheomelanin. Additional details about melanogenesis are available in Bolognia and Orlow (2003) and Meredith and Sarna (2006). The main steps involved in melanogenesis are depicted in Figure 1. It is important to note that eumelanin and pheomelanin seem to have different properties with respect to phototoxic and oxidizing potential, and this may explain the higher cancer risk observed in individuals with high pheomelanin contents (Hill and Hill, 2000; Takeuchi et al., 2004; Samokhvalov et al., 2005; Ye et al., 2006).

The regulation of melanogenesis and the distribution of melanin differ in the skin, hair, and iris. In the skin, the melanocytes located in the basal layer of the epidermis transfer melanosomes to adjacent keratinocytes through dentritic structures, and the keratinocytes eventually migrate to the upper layers of the epidermis (Rees, 2003). Within the keratinoctyes, melanosomes are typically aggregated over the nucleus, providing protection against ultraviolet radiation (UVR). In the hair, the hair bulb is the only site of melanin production. Here, highly melanogenic melanocytes transfer melanosomes to surrounding immature precortical keratinocytes that will eventually differentiate and migrate to form the pigmented hair shaft (Slominski et al., 2005). The epidermal-melanin unit (skin) and the follicular-melanin unit (hair) differ in some important ways. In the hair, melanogenesis only takes place during the anagen phase of the hair growth cycle (the period of hair shaft formation that lasts on average 3–5 years), while in the epidermis, it appears to be continuous (Ortonne and Prota, 1993). There seem to be additional differences related to the morphology of the melanocytes and the microenvironment in which melanogenesis takes place (Tobin and Bystryn, 1996; Tobin and Paus, 2001; Slominski et al., 2005). This may explain the discordant pigmentation patterns observed in some individuals (e.g., dark-pigmented hair and pale blue eyes and pale skin). In contrast to hair and skin, in the iris the melanosomes are found only within the melanocytes and the type of melanin and the density and distribution of melanosomes are the major determinants of eye color (Sturm and Frudakis, 2004). The iris contains two tissue layers. The thin inner layer is called the iris pigment epithelium (IPE) and does not contribute to normal iris color variation because the melanin in this heavily pigmented layer has a similar distribution in individuals with different iris colors (albinos are an exception). The outermost layer is

known as the iris stroma, and the melanin content in this layer is the primary cause of normal iris color variation (Sturm and Frudakis, 2004; Wielgus and Sarna, 2005).

Although there are differences in melanocyte density depending on body site (Whiteman et al., 1999), variation in the number of melanocytes does not seem to be a major factor explaining the pigmentation differences among human populations. Rather, differences in pigmentation are due to two major factors: the amount and type of melanin synthesized in the melanocytes (e.g., ratio of eumelanin to pheomelanin), and the shape and distribution of the melanosomes. Lightly pigmented skin is enriched in light-brown DHICA-eumelanin and yellow/red pheomelanins, and the melanosomes tend to be less pigmented, smaller in size, and packaged in groups. Darkly pigmented skin has more melanin, which is enriched in DHI-eumelanin (Alaluf et al., 2002). Additionally, the melanosomes of dark-skinned individuals are more pigmented, larger, and distributed as single units (Szabo et al., 1969; Alaluf et al., 2002). Figure 2 summarizes the population differences observed in the pattern of melanosome size and distribution in the skin. It has also been described that individuals with black hair have the highest eumelanin-to-pheomelanin ratio, those with blond or light brown hair show intermediate ratios, and those with red hair have the highest pheomelanin levels (Ortonne and Prota, 1993; Rees, 2003). With respect to eye color, brown irises have large amounts of melanin and high numbers of melanosomes, and absorb a substantial proportion of the incoming light, particularly at short wavelengths. In contrast, blue irises have low melanin content and few melanosomes. As a result of this, long-wavelength light penetrates the stroma and is absorbed in the IPE, while short-wavelength light (blue) is scattered by the collagen matrix of the iris. Green and hazel irises have intermediate amounts of melanin (Sturm and Frudakis, 2004).

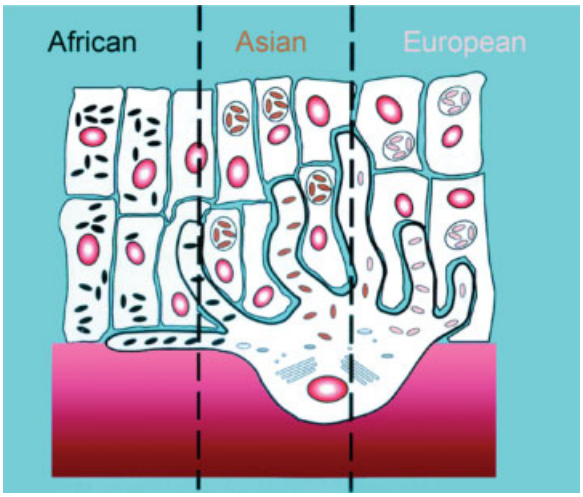

Fig. 2. Population differences in the pattern of melanosome size and distribution in the skin. Reproduced from Barsh, 2003. What controls variation in human skin color? *PLoS Biology* 1:19–22, with permission from PLoS Biology. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

MEASURING PIGMENTATION

The first attempts to use standardized methods to measure skin pigmentation were based on color-matching techniques. The most widely used was Von Luschan's chromatic scale, where a subject's skin pigmentation was compared with 36 small ceramic tiles ranging from white to black (Robins, 1991). However, these early methods, which were popular in the first half of the 20th century, had a high degree of subjectivity and were largely abandoned in the 1950s, when the first portable reflectance spectrometers became available for field work. Reflectance spectrometers measure the percentage of light reflected from the skin at different wavelengths and represented an important technological improvement in terms of objectivity and accuracy. The most widely used instrument was the EEL reflectance spectrophotometer (Evans Electroselenium Ltd., currently distributed by Diffusion Systems, UK), which measured the reflectance of the skin using nine filters corresponding to different wavelengths of the visible spectrum, from violet (601 filter, 425 nm) to deep red (609 filter, 685 nm). The readings of the instrument represented the reflectance of the skin at each wavelength relative to that of a white standard (typically white magnesium carbonate). Dozens of populations from around the world were sampled using the EEL reflectance spectrophotometer (although not always using all the available filters) from the time of its first use by Weiner (Weiner, 1951) for measuring pigmentation in 1951. More information about studies using the EEL reflectance spectrophotometer can be found in Byard (1981), Robins (1991), and Jablonski and Chaplin (2000). Other portable spectrometers were available to the scientific community, such as the Photovolt instrument. The Photovolt used principles similar to those of the EEL spectrophotometer, but the two instruments differed in the number and characteristics of the filters used to sample the visible spectrum: The EEL spectrophotometer had nine narrow wavelength filters while the Photovolt employed six filters with broader band transmittance. This made direct comparison of the results obtained with both instruments difficult, although conversion formulae were developed to overcome this issue (Lees and Byard, 1978; Lees et al., 1979). Currently, there are three strategies based on reflectance technologies to study the main chromophores of the skin (melanin and hemoglobin): tristimulus colorimetry, specialized narrow-band reflectometry, and diffuse reflectance spectroscopy. The modern instruments used to measure skin pigmentation offer significant advantages over previously available instruments in terms of portability and accuracy. In addition to these indirect approaches to measure pigmentation based on reflectometry, it is also possible to directly measure melanin content using biochemical methods. I briefly describe the available strategies to measure pigmentation below.

Tristimulus colorimetry

Tristimulus colorimetry was developed as a means of objectively representing color in a manner analogous to the way the eye perceives color (Hunter, 1942). The reflectance level of light through three particular broad wavelength filters (photodiode arrays on newer instruments) is determined. Color parameters are then defined by the levels of, and differences among, the reflectance levels of these three filters. The most commonly used color parameters are the Commission International d'Eclairage (CIE) $L^*a^*b^*$ system established in 1976. In

the CIELab color system, any color can be represented by three variables: L^*, the lightness-darkness axis; a^*, the red-green axis; and b^*, the blue-yellow axis, which can be plotted in three-dimensional space (Weatherall and Coombs, 1992). Tristimulus colorimeters like the Minolta Chroma Meter series (Konica Minolta Sensing, Osaka, Japan) are usually able to report color values for a number of other color systems as well. In studies using tristimulus colorimeters, erythema (redness of the skin primarily due to hemoglobin) is typically evaluated using the a^* parameter, and pigmentation is evaluated using L^*, b^*, or a combination of the three parameters (Stamatas et al., 2004).

Specialized narrow-band reflectometry

This strategy is based on the work of Diffey et al. (1984), who developed a method to specifically measure hemoglobin and melanin in the skin. Hemoglobin is present in the blood vessels of the dermis in two major forms, deoxy-hemoglobin (hemoglobin with the oxygen-binding sites unoccupied by oxygen) and oxy-hemoglobin (oxygen-binding sites occupied by oxygen molecules). Hemoglobin primarily absorbs in the lower wavelengths of visible light, with typical absorption peaks in the green-yellow region of the spectrum (deoxy-hemoglobin absorption maxima, 555 nm; oxy-hemoglobin absorption maxima, 540 and 577 nm), and absorbs very little in the red wavelengths, which is why blood is red (Kollias and Stamatas, 2002). In contrast, melanin shows absorbance of light of all wavelengths. Melanin's absorption decreases exponentially in the range 400–600 and then linearly in the range of 720–620 nm (Kollias et al., 1991). Based on these differences in the spectral curves of hemoglobin and melanin, Diffey et al. (1984) suggested that the reflectance of narrow-band light in the red spectrum would yield reasonable estimates of the melanin content of the skin, following the equation:

$$M\,(\text{melanin index}) = \log_{10}\left(\frac{1}{\%\,\text{red reflectance}}\right)$$

The degree of skin redness or erythema can be calculated by subtracting the absorbance due to melanin from the absorbance of the green filter and is calculated as:

$$E\,(\text{erythema index}) = \log_{10}\left(\frac{1}{\%\,\text{green reflectance}}\right) - \log_{10}\left(\frac{1}{\%\,\text{red reflectance}}\right)$$

A number of narrow-band spectrometers that provide measurements of the melanin and erythema indices are commercially available, including the DermaSpectrometer (Cortex Technology, Hadsund, Denmark), the Erythema/Melanin Meter (DiaStron, DiaStron, Hampshire, UK), and the Mexameter (Courage-Khazaka Electronic, Köln, Germany). Shriver and Parra (2000) carried out a comparative study of hair and skin pigmentation in individuals of diverse ancestry using a tristimulus colorimeter (Photovolt ColorWalk) and a narrow-band spectrometer (DermaSpectrometer). They showed that there is a high correlation between L^* and M, the parameters typically used to measure melanin content, and concluded that, while both instruments provide accurate estimates of melanin levels in skin and hair, measurements using narrow-band instruments may be less affected by the greater redness of certain body sites due to increased vascularization.

Diffuse reflectance spectroscopy

Diffuse reflectance spectroscopy (DRS) is considered the most versatile and accurate noninvasive strategy to measure skin pigmentation (Stamatas et al., 2004). In contrast to the two methods described before, which are based on reflectance values in a subset of the visible spectrum, DRS reflectance values are measured at high optical resolution throughout the full visible spectrum. Currently, several companies manufacture portable spectrometers at reasonable prices, including OceanOptics (Boca Raton, FL), Newport (Irvine, CA), and Stellarnet (Tampa, FL). The illumination light is delivered to the skin at the site of interest using a bifurcated fiber optic bundle or an integrating sphere. An analyzer-detector system (typically a diffraction grating coupled to a detector array) allows simultaneous acquisition of the whole spectrum. Figure 3 shows an example of skin reflectance curves from 12 individuals of diverse ancestry measured on the inner upper arm at 0.5 nm intervals along the visible spectrum (400–700 nm) using a Stellarnet EPP2000 reflectance spectrometer (E.J. Parra, unpublished data). The availability of data from the full visible spectrum means that it is possible to separate the effects of the two major chromophores of the skin, melanin and hemoglobin, because of their different spectral properties. Kollias and Baqer (1985, 1987) have shown that the amount of melanin in the skin can be estimated by DRS from the slope of the absorbance spectrum in the region between 620 and 720 nm. The amount of hemoglobin can be determined by considering the absorbance of the skin at 560–580 nm, where absorption by hemoglobin is more prominent, corrected by the melanin absorption spectrum (Stamatas et al., 2004). An additional advantage of the new generation of portable spectrometers is that they can be used to calculate all types of pigmentation measures, such as CIELab and other standard color systems, and the reflectance levels at narrow-bands (such as those used by the DermaSpectrometer) can be determined using appropriate formulae.

Biochemical measurements of melanin content

As mentioned above, pigmentation can be studied using noninvasive techniques based on reflectometry. However, using these techniques it is not possible to directly quantify the three major types of melanin: yellow-red pheomelanin, light brown DHICA-enriched eumelanin, and dark brown-black DHI-enriched eumelanin. The most common strategy for directly determining melanin composition entails the measurement of chemically degraded melanin by-products using high-performance liquid chromatography (HPLC). Pheomelanin and DHICA-enriched eumelanin contents can be determined using HPLC, and the amount of DHI-enriched eumelanin can be estimated as the difference between the total melanin content, measured using spectophotometry, and the amounts of pheomelanin and DHICA-enriched melanin, measured using HPLC (Alaluf et al., 2001). HPLC-based methods have been used to estimate eumelanin and pheomelanin contents in human hair and skin (Ito

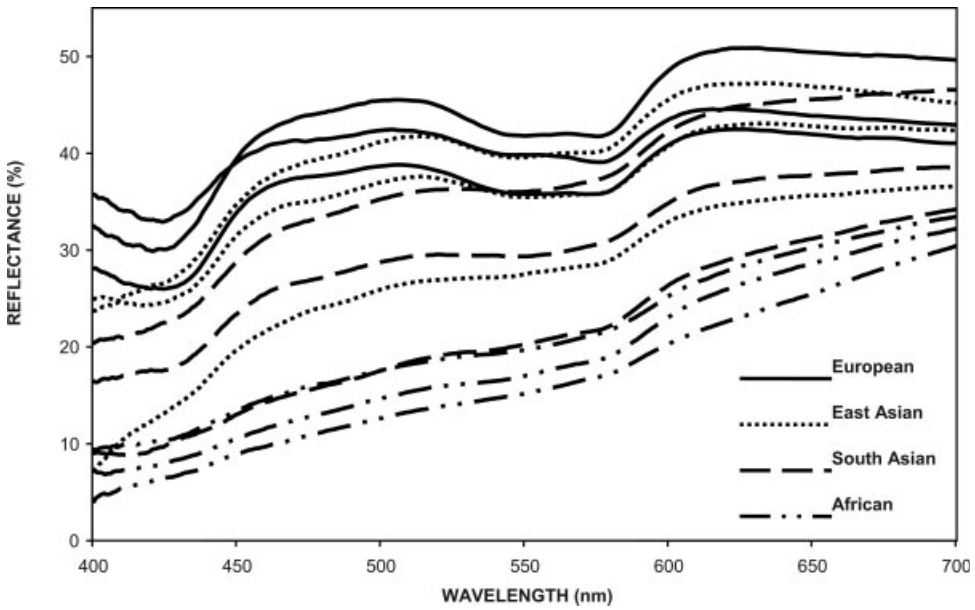

Fig. 3. Skin reflectance curves for individuals of European, East Asian, South Asian, and African ancestry. Skin reflectance was measured on the inner upper arm at 0.5-nm intervals along the visible spectrum (400–700 nm) using a Stellarnet EPP2000 reflectance spectrometer. Note the decrease in skin reflectance in the green-yellow wavelengths (540–580 nm) due to hemoglobin absorption, which is particularly evident in the curves of the individuals with the highest skin reflectance.

and Fujita, 1985; Thody et al., 1991; Ozeki et al., 1996; Alaluf et al., 2001). The major disadvantage of this approach for determining pigment levels in the skin is that it is an invasive method, requiring suction blisters or punch biopsies. There have been comparative studies showing that there is a high correlation between epidermal melanin content estimated using biochemical and reflectance methods (Alaluf et al., 2002, Kongshoj et al., 2006).

Although methods based on reflectance technologies have gained widespread acceptance during the last decade, alternative strategies are still widely used in some fields. For example, the skin classification system developed in 1975 by Thomas B. Fitzpatrick and coworkers is still widely used today in the field of dermatology (Fitzpatrick, 1988). This system involves the classification of a person into one of six sun-reactive skin types (I, always burns/never tans; II, usually burns/tans less than average; III, sometimes mildly burns/tans average; IV, rarely burns/tans more than average; V, never burns/brown skin; VI, never burns/black skin) (Fitzpatrick, 1988). This scale has been criticized for various reasons, including its poor predictive value, poor correlation with skin color, and poor correlation with UV sensitivity (Westerhof et al., 1990; Leenutaphong, 1995; Snellman et al., 1995; Damian et al., 1997).

I would like to finish this section devoted to the techniques available for the measurement of pigmentation by discussing strategies used to determine iris color. The conventional methods based on reflectometry or biochemical analysis cannot be applied to evaluate iris pigmentation in anthropological or genetic studies. Traditionally, studies of iris color have been based on classification of iris pigmentation in broad categories (e.g., blue, gray, green, hazel, light brown, dark brown) by trained observers (Eiberg and Mohr, 1996; Rebbeck et al., 2002; Zhu et al., 2004; Duffy et al., 2007). However, this qualitative approach fails to capture the quantitative nature of iris pigmentation variation. Recently, there have been attempts to develop quantitative methods to estimate iris color based on photographs taken under standardized conditions. German et al. (1998) and Niggemann et al. (2003) reported iris pigmentation in the Red-Green-Blue (RGB) color space, and Melgosa et al. (2000) quantified iris color using the CIELab color system. More recently, Fan et al. (2003) have developed a method that automatically extracts the iris region from photographs, computes the iris color, and corrects the color based on a standard calibration target. These and other studies are paving the way for much more objective and precise measurement of eye color, which will allow quantitative evaluation of even subtle pigmentation variation within the iris (e.g., darker pigmentation in the peripupillary area).

DISTRIBUTION OF PIGMENTATION IN HUMAN POPULATIONS

The distribution of skin pigmentation differs from that of other phenotypic traits and most genetic markers. Relethford (2002) estimated that 88% of the total skin pigmentation variation can be explained by pigmentation differences among major geographic groups. This value stands in sharp contrast to what has been described in numerous autosomal genetic studies, which indicate that for an "average" genetic marker in humans, variation among major geographic groups typically accounts for only 10–15% of the total diversity (Lewontin 1972; Jorde and Wooding, 2004; Tishkoff and Kidd, 2004). Other quantitative traits, such as craniometric traits, show a pattern of diversity broadly consistent with the genetic data; only 13% of the total variation is due to differences among major geographic regions (Relethford, 2002). Clearly, skin pigmentation shows an atypical distribution, especially considering the recent origin of anatomically modern humans (~200,000 years ago, Foley, 1998). Figure 4 shows the global distribution of skin pigmentation, based on the map of the Italian geographer Renato Biasutti. Biasutti (1953) did not use reflectometry to measure skin pigmentation (his research took place in the first half of the 20th century) and his sampling of

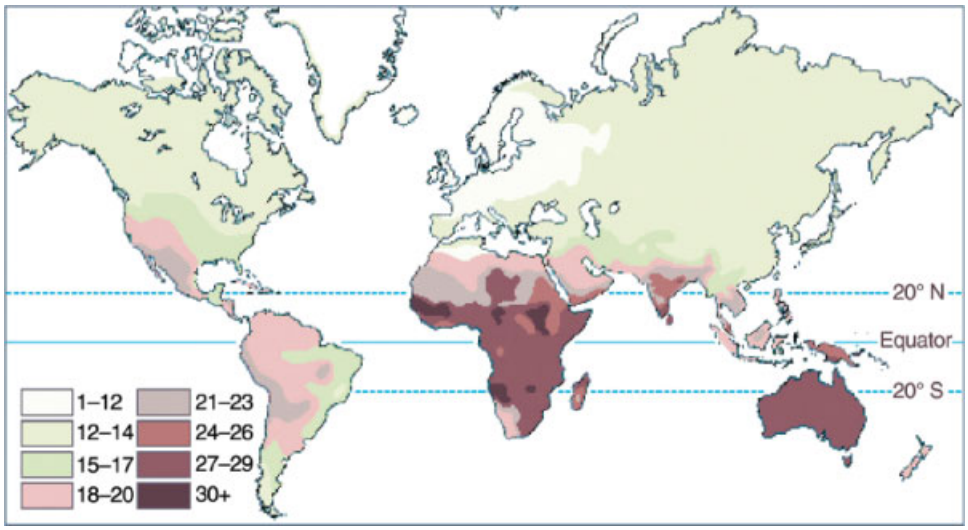

Fig. 4. World map showing skin pigmentation distribution. The map is based on the work of the Italian geographer R. Biasutti. Higher numbers represent darker skin color. Source: D. O'Neil (Behavioral Sciences Department, Palomar College, San Marcos, CA; http://anthro.palomar.edu/vary/vary_1.htm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

human populations was not comprehensive. However, Biasutti's map is still useful to describe the general geographic patterns of skin pigmentation in human populations, which show a strong correlation with latitude. Skin color tends to be darker in equatorial and tropical areas (sub-Saharan Africa, South Asia, Australia, and Melanesia) than in areas located far from the equator (see Fig. 4). The correlation of skin pigmentation and latitude was also demonstrated in a study carried out by Relethford (1997). Importantly, Relethford used more precise pigmentation estimates based on reflectometry, and he found a strong relationship of skin reflectance with latitude (see Fig. 5). The underlying factor explaining this remarkable relationship between skin pigmentation and latitude seems to be UVR intensity, which is greater at the equator and progressively diminishes with increasing latitude. More recently, Jablonski and Chaplin (2000) published a comprehensive study, which evaluated the relationship of skin reflectance measurements in populations throughout the world with UVR levels estimated using remote sensing technology. These authors observed a strong correlation between skin reflectance and UVR levels and found an excellent agreement of the skin reflectance values predicted by a regression model using average UVR as an independent variable with the observed reflectance values. In summary, the available body of data clearly indicates that the distribution of skin pigmentation in humans has been strongly influenced by UVR levels. I will discuss this issue in more detail in the next section, which is devoted to the evolution of human skin pigmentation.

In contrast to skin pigmentation, which shows a strong correlation with latitude, variation in hair and iris color is much more restricted geographically. Most human populations have dark hair and irises. Red and blond hair are mainly found in European populations (the highest frequencies of red hair occur in Great Britain and Ireland and the highest frequencies of blond hair in Nordic countries), although blondism is a trait commonly found in some Australian and Melanesian populations (Birdsell, 1993). Similarly, the lighter iris colors (blue, green, and hazel) are primarily found in European populations, although they are also present in Middle Eastern, North African, West Asian, and South Asian populations (Sturm and Frudakis, 2004).

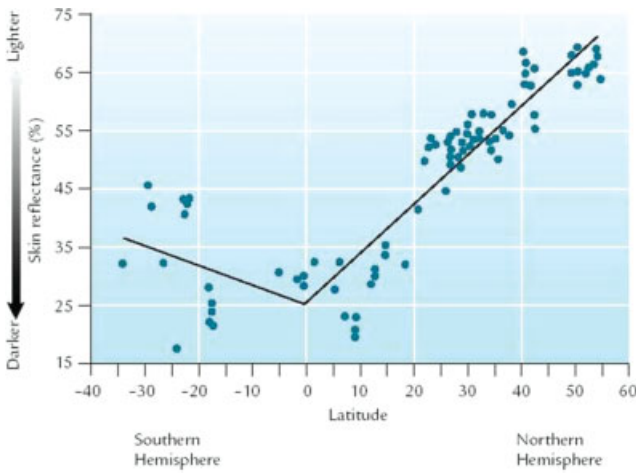

Fig. 5. Relationship of skin reflectance with latitude. Source: John Relethford (The human species, 6th ed., McGraw-Hill, 2005, p. 182), reproduced with permission of the McGraw-Hill Companies. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

HYPOTHESES REGARDING THE EVOLUTION OF SKIN PIGMENTATION IN HUMANS

Numerous hypotheses have been put forward to explain the evolution of skin pigmentation in human populations, taking into account the observed relationship between melanin levels and latitude. In this section, I briefly discuss the major hypotheses. Additional information can be found in Robins (1991) and Jablonski (2004). According to most authors, the underlying factor explaining the unique geographic distribution of skin pigmentation in humans appears to be UVR exposure (Relethford, 1997, 2002; Jablonski and Chaplin, 2000 and references therein). Melanin acts as a key photoprotective layer in the skin. Of particular importance is melanin's role in filtering the harmful UVR from the sun (280–400 nm). Additionally, melanin (in particular eumelanin) also exerts a protective effect by scavenging reactive free radicals and other oxidants (Bustamante et al., 1993; Krol and Liebler, 1998). There are several

potential selective factors that could have driven the evolution of highly melanized skin in equatorial and tropical regions with high incidence of UVR.

Melanin and protection from sunburn and skin cancer

Melanin acts as a natural sunblock and is especially effective in protecting against the harmful effects of the shorter wavelengths of the electromagnetic radiation (~300 nm), which are the most damaging to DNA and proteins (Rees, 2003). The initial effect of skin exposure to UVR is sunburn, which is characterized by erythema (redness), edema, and possibly pain and blistering (Rees, 2004). Severe sunburn can cause damage to the sweat glands, which can lead to a disruption in thermoregulation, and can also result in an increased risk of infection in damaged skin cells (Jablonski and Chaplin, 2000; Rees, 2004). Therefore, in the tropical environments in which our species first evolved, dark skin with high amounts of UVR absorbing eumelanin would have been highly advantageous, while lighter skin that was prone to sunburn, damaged sweat glands, and infections would have been selected against. It is also well known that extended exposure to the sun can lead to skin cancer when UVR damages genes that normally inhibit cancerous growth (Halliday, 2005; Ullrich, 2005). There is evidence indicating that there are major differences in skin cancer risk depending on skin type, with dark skin being much less susceptible to several types of skin cancer (Rees, 2004). For this reason, some authors have indicated that natural selection favored darkly pigmented skin in tropical and equatorial regions to protect against skin cancer (Robins, 1991 and references therein). However, others argue that since some types of skin cancer (basal and squamous cell carcinomas) are rarely fatal and since most individuals do not develop cancer (including malignant melanomas, the most serious type of skin cancer) until they are past their reproductive years, it is unlikely that skin cancer had a significant role in the evolution of skin color (Jablonski and Chaplin, 2000). In this respect, it is important to mention that albinos living in areas with high incidence of UVR (e.g., sub-Saharan Africa) typically develop premalignant lesions or skin cancer as teenagers or in early adulthood, and in Nigeria and Tanzania, less than 10% of albinos survived beyond the age of 30 (Robins, 1991; Yakubu and Mabogunje, 1993; Rees, 2003).

Melanin and protection against nutrient (folate) photolysis

Sunlight is not only damaging to human skin, but also to some essential nutrients, particularly folate. Folate is necessary for DNA synthesis and repair, and folate deficiency can result in complications during pregnancy and multiple fetal abnormalities, including neural tube defects, such as spina bifida and anencephalus (Lucock, 2000; Off et al., 2005). Folate deficiency was an important cause of perinatal and postnatal mortality in some populations before the introduction of preventive supplementation (Jablonski and Chaplin, 2000). Folate also plays a key role in spermatogenesis (Mathur et al., 1977; Cosentino et al., 1990; Wong et al., 2002; Ebisch et al., 2006). Central to this discussion is the fact that folate is extremely sensitive to UVR (Off et al., 2005). Branda and Eaton (1978) found that folate concentrations in human plasma decreased significantly after brief exposure to UVR and they also observed that light-skinned patients undergoing therapeutic UVR exposure had lower folate levels than controls. This suggests that in geographic areas with high levels of UVR, light-skinned individuals will be more prone to folate deficiency than those with dark skin. Due to the important role of folate in several key biological processes, it is likely that maintenance of optimal folate levels has been under the influence of natural selection.

It is important to mention that the two hypotheses described above are compatible and either singly or in combination can explain the eumelanin-rich, dark pigmentation observed in regions with high UVR incidence. The genetic data available for some pigmentation candidate genes (e.g., *MC1R*) are consistent with natural selection favoring dark pigmentation in tropical areas, and will be discussed in more detail in the next section. However, these hypotheses are insufficient to explain the global distribution of skin pigmentation (Figs. 4 and 5). In order to explain the strong correlation of skin pigmentation with latitude (or UVR), it is necessary to clarify the evolutionary factors responsible for the lightening of the skin in regions with low UVR. I summarize the two main hypotheses below.

Melanin and vitamin D synthesis

Although the effects of UVR on the skin are for the most part harmful, there is one important exception: UVB radiation is essential for the synthesis of vitamin D in the skin. While some dietary sources have substantial amounts of vitamin D (particularly fatty fish and fish oil, egg yolk, and organ meats), cutaneous synthesis of vitamin D via exposure to the sun is the main source of vitamin D (Holick, 2003, 2005). Vitamin D plays a key role in bone metabolism and vitamin D deficiency results in rickets in children and osteomalacia in adults. In recent years, other functions of vitamin D have been recognized, including immunoregulation and regulation of cell differentiation and proliferation (Holick, 2004).

According to the original version of the vitamin D hypothesis advanced by Loomis (1967), the correlation between skin pigmentation and latitude is the result of selection favoring dark skin near the equator in order to prevent an excess of cutaneous synthesis of vitamin D, which could be potentially toxic, and selection favoring light skin far from the equator in order to maximize vitamin D synthesis in regions with low UVR incidence. Loomis' views on vitamin D toxicity have since then been disproved, as it has been demonstrated that sun exposure does not result in vitamin D toxicity (Holick, 2007). The current versions of the vitamin D hypothesis explain the modern distribution of human pigmentation as the result of a balance between natural selection favoring protection against sunburn and folate destruction in areas where UVR exposure is high (see earlier), and selection for lighter pigmentation in regions far from the equator in order to facilitate vitamin D synthesis. People with dark skin (e.g., Fitzpatrick's skin phototype V/VI) require at least 10 times more exposure to sunlight than those with light skin (e.g., skin phototype II/III) to produce the same amount of vitamin D in their skin (Holick, 2003). This suggests that individuals with darker skin would be at a selective disadvantage in synthesizing vitamin D in areas of reduced UVR. Some authors (e.g., Robins, 1991) have cast doubt on the valid-

ity of the vitamin D hypothesis, mainly on the grounds that in high latitude regions, synthesis of vitamin D during the spring and summer months would be sufficient to produce stores of vitamin D in fat and muscle to secure adequate levels of vitamin D in the winter. However, the analysis of UVR levels using remote sensing technology (Jablonski and Chaplin, 2000) indicates that there is a broad region in the Northern Hemisphere where there is not enough UVR to permit adequate cutaneous synthesis of vitamin D, even in lightly pigmented skin (see Fig. 6 for map corresponding to worldwide UVR zones, based on the analysis of Jablonski and Chaplin). Importantly, Robins' assertion that vitamin D synthesis in the spring and summer months would be sufficient to maintain adequate levels of vitamin D during the winter is contradicted by numerous clinical studies indicating that vitamin D insufficiency is present in epidemic proportions at high latitudes, and that dark-skinned populations are at particular risk of vitamin D insufficiency (Calvo and Whiting, 2003; Holick, 2005). In fact, there is research indicating that vitamin D levels are much lower throughout the year in dark-skinned than light-skinned individuals living at high latitudes, and these lower vitamin D levels cannot be explained by differences in vitamin D intake (Harris and Dawson-Hughes, 1998). This issue will be discussed in more detail in the section Implications of pigmentation for public health. Overall, the vitamin D hypothesis seems to be a reasonable explanation for the lower melanin levels observed at high latitudes, although other evolutionary factors may also have played a role (see following). It is quite possible that the major selective mechanism was the well-known effect of vitamin D on bone growth and development (Beall and Steegmann, 2000). However, it is important to note that research carried out in the last decade clearly indicates that the role of vitamin D goes well beyond calcium homeostasis. Vitamin D receptors are present in kidney, keratinocytes, osteoblasts, activated T and B lymphocytes, β-islet cells, small intestine, prostate, colon, and most organs in the body (including brain, heart, skin, gonads, prostate and breast) and vitamin D plays an important role in the prevention of autoimmune diseases, control of invading pathogens, and regulation of cell growth and differentiation (MacDonald, 1999; Hansen et al., 2001; Omdahl et al., 2002; Holick, 2003, 2004; Zasloff, 2006).

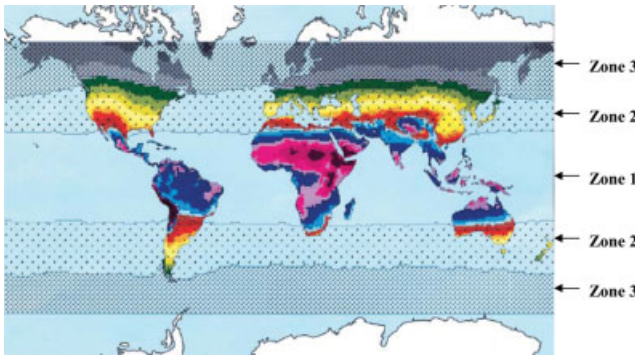

Fig. 6. Map showing the potential for synthesis of previtamin D_3 in lightly pigmented human skin, computed from annual average UVMED values (which are indicated with different colors in the online version of the article, and different shades of gray in the printed version). UVMED is the quantity of UV radiation (UVR) required to produce a barely perceptible reddening of lightly-pigmented skin. In zone 1, in the tropics, there is adequate UVR throughout the year to produce previtamin D_3. In zone 2 (light stippling), there is insufficient UVR during at least 1 month of the year to produce previtamin D_3 in the skin. In zone 3 (heavy stippling), there is not enough UVR for previtamin D_3 synthesis on average for the whole year. Reprinted from Jablonski and Chaplin, 2000. The evolution of human skin coloration. *Journal of Human Evolution* 39:57–106, with permission from Elsevier. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Sexual selection

The idea that sexual selection could be responsible for skin color variation has a long history. In his book *The descent of man*, Charles Darwin (1871) stated that the population differences observed for several human traits, including pigmentation, could be the result of sexual selection. Indeed, there is evidence indicating that pigmentation is an important criterion of mate choice in humans (Aoki, 2002 and references therein), leading some authors to postulate that sexual selection has been an important factor shaping the distribution of not only skin, but also hair and eye color in humans (Diamond, 1991; Aoki, 2002, Frost et al., 2006). Similarly, the observation that females tend to be lighter than males in most populations has been interpreted as a result of sexual selection (Van den Berghe and Frost, 1986, Frost, 1994), although this sexual dimorphism has been interpreted by other authors to be a result of natural selection, because females have higher calcium requirements than males and this would favor lighter skin pigmentation in order to increase vitamin D synthesis (Jablonski and Chaplin, 2000). Of course, natural selection and sexual selection are not mutually exclusive processes, and it is possible that both have shaped the current distribution of pigmentation in human populations, either as primary or secondary factors. This possibility has been suggested by multiple authors (Cavalli Sforza et al., 1994; Frost, 1994; Aoki, 2002; Jablonski and Chaplin, 2000, among others), although some authors put more emphasis on natural selection and assign sexual selection a secondary role (Jablonski and Chaplin, 2000), while others believe that sexual selection had a more prominent role (Diamond, 1991). In 2002 Aoki reformulated the sexual selection hypothesis to explain the global distribution of skin pigmentation in humans. According to Aoki (2002: p. 592), the observed gradient of skin pigmentation is due to "1) ubiquitous natural selection against light skin that is strong near the equator, becomes progressively weaker at higher latitudes, and is perhaps negligible at the limits of human habitation; and 2) sexual preference for light skin that is everywhere of roughly the same intensity." Aoki (2002) criticized the vitamin D hypothesis, using arguments similar to those used by Robins (1991) and based his argument for sexual selection primarily on a study by Van den Berghe and Frost (1986), indicating that there is a preference for a lighter-than-average skin color in 47 out of 51 societies, and that the preference for lighter skin color is more strongly expressed in males. Madrigal and Kelly (2006) recently tested, using reflectance data, whether there is a positive correlation between increasing distance from the equator and increased sexual dimorphism, as predicted from Aoki's hypothesis, and they found no evidence to support his prediction. In my judgment, the photoprotection and vitamin D hypotheses satisfactorily explain the general latitudinal gradient observed in human skin pigmentation

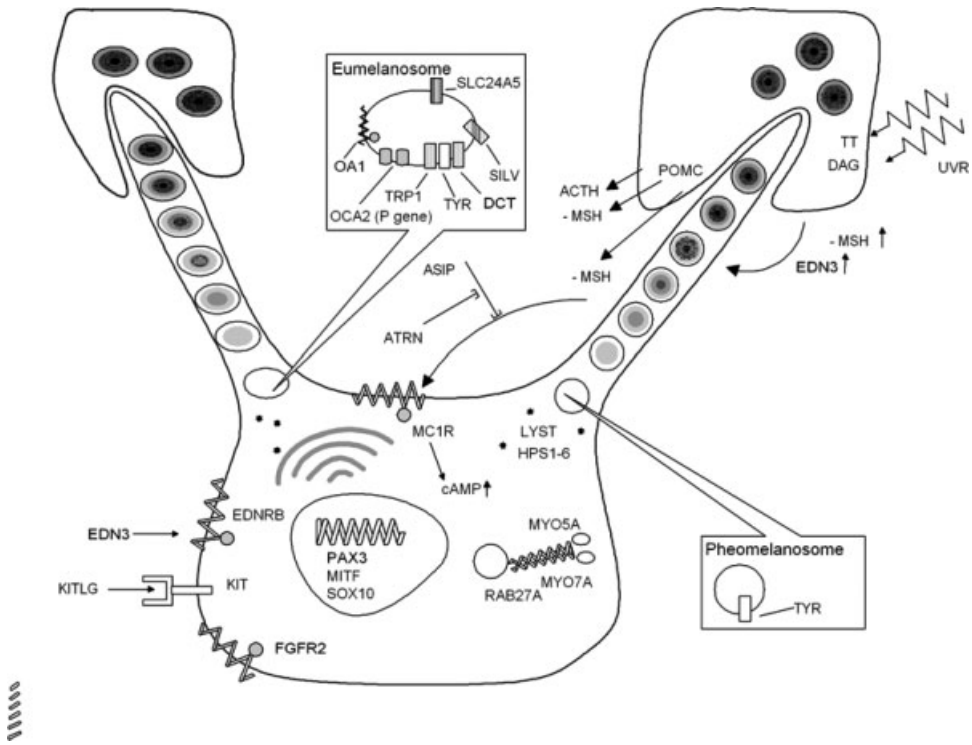

Fig. 7. Graphical representation of a melanocyte showing some important proteins involved in melanocyte development and the pigmentation pathway, including the following: 1) the tyrosinase enzyme complex (TYR, TRP1, DCT) located on the membrane of the melanosome, which is responsible for the enzymatic conversion of the amino acid tyrosine into melanin; 2) other proteins located within the melanosomes that play a critical role in melanogenesis (MATP, P gene, SILV, SLC24A5); 3) the signaling pathways affecting the regulation of melanin synthesis, including hormones and receptors (α-MSH, MC1R, ASIP, ATRN); 4) the transcription factors involved in melanin production (PAX3, MITF, SOX10); 5) proteins involved in melanosome transport (MYO5A, MYO7A, RAB27A) and melanosome construction/protein routing (CHS1, HPS1-6); and 6) developmentally important ligands (EDN3, KITLG) and receptors (KIT, EDNRB) which control melanoblast migration and differentiation. Modified from Heather Norton and Mark Shriver, with permission (personal communication, August 2007).

and Aoki's hypothesis, which requires a universal preference for light skin color, both in time and space, has limited support. However, this does not exclude the possibility that sexual selection has shaped, to an extent, the pigmentation variation observed in human populations.

In closing this section, it is necessary to mention that there are other hypotheses explaining the distribution of skin pigmentation in humans. For example, Wassermann (1965) and more recently MacKintosh (2001) linked dark skin pigmentation with resistance to bacterial, parasitic, and viral infections, and Post et al. (1975) suggested that depigmented skin would provide resistance to cold injury.

THE GENETIC BASIS OF NORMAL PIGMENTATION VARIATION

In spite of recent breakthroughs in our knowledge of the pigmentary system, and the physiological and evolutionary importance of pigmentation, very little is known about the genetic basis of normal variation in human pigmentation (Barsh, 2003). The pigmentation of unexposed areas of the skin (constitutive pigmentation) is fairly stable throughout the life of an individual and does not change much due to environmental factors (Robins, 1991). Constitutive pigmentation is a polygenic trait, but the number of genes and the exact nature of the allelic variants determining melanin content remain, for the most part, unknown. Studies of human pigmentation disorders, combined with studies of animal pigmentation models, have greatly increased our knowledge of the pigmentary system. These studies have brought a new understanding of the diverse pathways driving melanin production and regulation, including the following: 1) the tyrosinase enzyme complex (TYR, TRP1, DCT) located on the membrane of the melanosome, which is responsible for the enzymatic conversion of the amino acid tyrosine into melanin; 2) other proteins located within the melanosomes that play a critical role in melanogenesis (MATP, P gene, SILV, SLC24A5); 3) the signaling pathways affecting the regulation of melanin synthesis, including hormones and receptors (α-MSH, MC1R, ASIP, ATRN); 4) the transcription factors involved in melanin production (PAX3, MITF, SOX10); 5) proteins involved in melanosome transport (MYO5A, MYO7A, RAB27A) and melanosome construction/protein routing (CHS1, HPS1-6); and 6) developmentally important ligands (EDN3, KITLG) and receptors (KIT, ENDRB), which control melanoblast migration and differentiation. Figure 7 shows a representation of a melanocyte indicating some of the proteins involved in the pigmentary system. A more complete list of the genes involved in pigmentation is available at http://albinismdb.med.umn.edu/genes.htm. Comprehensive reviews about the pigmentary system and the genetics of pigmentation can be found in Barsh (1996, 2003), Nordlund et al. (1998), and Sturm et al. (2001). Given the polygenic inheritance of pigmentation, and the complexity of the pigmentary

system, it is not surprising that it has been very challenging to identify the genes responsible for the variation observed in skin, hair, and iris pigmentation. However, the situation has changed dramatically in the last decade, and several genes have been associated with normal pigmentation variation. I summarize the available evidence below, and also discuss novel strategies of data analysis taking advantage of the large-scale catalogs of genome variation (Hinds et al., 2005; The International HapMap Consortium 2005) that will undoubtedly advance our understanding of pigmentation variation in years to come.

Genes involved in normal variation in skin, hair, and eye color in human populations

MC1R. The melanocortin 1 receptor gene (*MC1R*) is without a doubt the most exhaustively studied pigmentation candidate gene. The role of this gene in normal human pigmentation was clarified by studies showing an association of variants of *MC1R* with red hair and fair skin (Valverde et al., 1995; Bastiaens et al., 2001; Schaffer and Bolognia, 2001; Naysmith et al., 2004). The melanocortin 1 receptor is a member of the family of G protein-coupled receptors known as melanocortin receptors and has a critical role in switching between eumelanin and pheomelanin synthesis in the melanocytes. Binding of the melanocyte-stimulating hormone (α-MSH) to MC1R results in activation of adenylyl cyclase, and increased levels of intracellular cAMP and tyrosinase activity. The ultimate outcome is the production of eumelanin within the melanocytes. Conversely, when the antagonist agouti signaling protein (ASIP) binds to MC1R, there is a decrease in tyrosinase activity and a switch to pheomelanin production. The *MC1R* gene shows an interesting pattern of polymorphism worldwide (for a recent review, see Makova and Norton, 2005). Rana et al. (1999) and Harding et al. (2000) sequenced this gene in samples from several continents, and they found a surprising lack of diversity in sub-Saharan African populations; in particular, nonsynonymous variants were absent in all the African samples analyzed. Similarly, the frequency of amino acid variants in other dark-skinned populations, such as Papuans and South Asians, was very low. This lack of diversity in dark-skinned populations can be explained as a result of a strong functional constraint on *MC1R*. Although a recent study by John et al. (2003) reported three nonsynonymous mutations in individuals from South Africa (farther south of the equator than the previous African samples analyzed), the overall picture is consistent with the action of purifying selection removing *MC1R* mutations that could promote pheomelanin synthesis in sub-Saharan Africa and possibly in other regions with high UVR incidence (Makova and Norton, 2005). The situation is very different in Europe, East Asia, and Southeast Asia, where *MC1R* is highly polymorphic. In fact, the levels of nucleotide diversity observed in the *MC1R* gene in these populations are higher than the values observed in other autosomal genes (Makova and Norton, 2005). More than 30 alleles have been reported in European populations, with more than 20 nonsynonymous variants (Sturm et al., 2001). Importantly, at least nine nonsynonymous variants are present at frequencies of 1% or higher in European populations. Four of these mutations have a strong association with the red hair/fair skin phenotype (Asp84Glu, Arg151Cys, Arg160Trp, and Asp294His), while three show only a weak association (Val60Leu, Val92Met, and Arg163Gln) (Duffy et al., 2004). *In vitro* studies have shown that some of these variants have reduced ability to bind α-MSH (e.g., Val92Met) or to activate adenylyl cyclase (e.g., Arg151Cys, Arg160Trp, and Asp294His) (Xu et al., 1996; Frändberg et al., 1998; Schiöth et al., 1999). This explains the association of these variants with hair and skin phenotypes characterized by a rich content in pheomelanin. It is important to mention that individuals harboring these functional variants burn easily and have poor tanning capacity, and numerous studies have indicated that these variants increase the risk of melanoma and nonmelanoma skin cancers (reviewed in Rees, 2004). Similarly, studies in cultured melanocytes expressing these functional variants of *MC1R* show a decrease in eumelanin production and an increased sensitivity to the cytotoxic effects of UVR (Scott et al., 2002). The importance of skin type and *MC1R* variants in skin cancer risk will be further discussed in the section Implications of pigmentation for public health. The variation of the gene *MC1R* in Asian populations has not been studied as extensively as in European populations, but the available data indicate high levels of polymorphism in East Asia and Southeast Asia (Rana et al., 1999; Harding et al., 2000; Yao et al., 2000; Peng et al., 2001; Nakayama et al., 2006). Asian populations are characterized by high frequencies of two nonsynonymous variants, Arg163Gln and Val92Met, which are also present at much lower frequencies in European populations (see Nakayama et al., 2006 for a review of *MC1R* allele frequencies in East and Southeast Asia). Interestingly, Nakayama et al. (2006) have recently described three novel functional variants showing very dramatic reduction in MC1R activities. These variants are restricted to high latitudes, suggesting once again that adaptation to UVR levels may have played an active role in shaping the distribution of *MC1R* polymorphisms.

SLC24A5. The "golden" gene (*SLC24A5*) has been the target of selection during the evolutionary process that resulted in the lightening of the skin of European (and closely related) populations (Lamason et al., 2005). The role of this gene in human pigmentation variation was identified in a somewhat atypical fashion; the story began with researchers working with zebrafish (*Danio rerio*), a model organism used in laboratories around the world. One of the zebrafish mutants, the so-called *golden* mutant, is characterized by a delayed and reduced development of pigmentation with respect to the wild-type zebrafish (see Fig. 8). Using linkage analysis combined with morpholino-knockdown experiments, Lamason et al. (2005) identified *slc24a5* as the gene responsible for the zebrafish *golden* mutant. The golden phenotype is due to a premature stop codon that truncates the protein, resulting in lightening of the pigmented stripes of the zebrafish. Interestingly, this protein is conserved in other vertebrates and it was possible to rescue melanin pigmentation by injecting human mRNA in *golden* zebrafish embryos. The golden gene encodes a cation exchanger that seems to have a critical role in melanosome morphogenesis and melanogenesis. When the authors retrieved the information available at the HapMap database (http://www.hapmap.org) about the human *SLC24A5* gene, they found a very unusual pattern of polymorphism. Several SNPs, including a nonsynonymous polymorphism (rs1426654) encoding alanine or

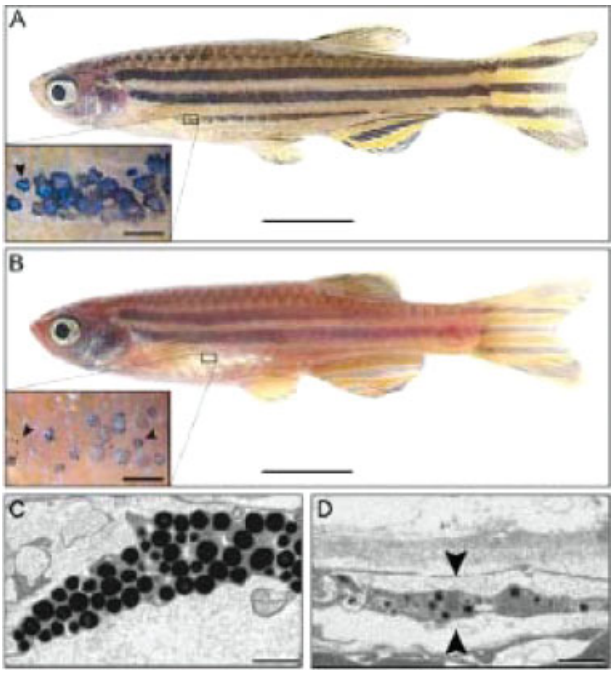

Fig. 8. Photographs showing the differences in pigmentation between the adult wild-type zebrafish (**A**) and the *golden* zebrafish (**B**). Note the lighter stripes in the *golden* zebrafish. The melanophores of the golden zebrafish (**D**) are thinner and contain fewer melanosomes than do those of the wild-type (**C**). Source: Lamason et al., 2005. *Science* 310:1782–1786, with permission from AAAS. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley. com.]

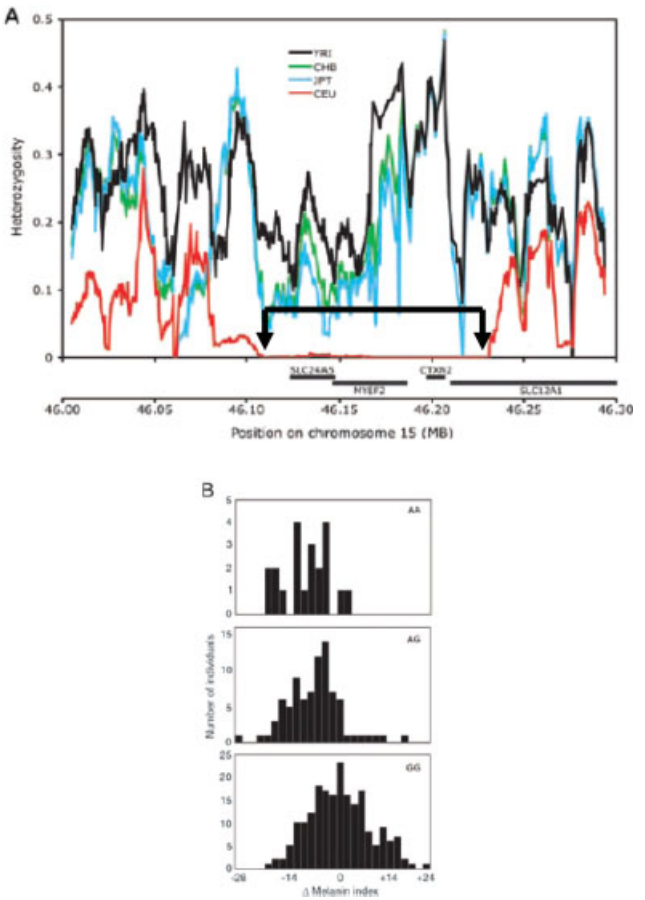

Fig. 9. **A**: Evidence of a selective sweep in the region of the *SLC24A5* gene in European populations. Note the dramatic reduction of heterozygosity in the European sample in the region encompassing the *SLC24A5* gene (enclosed by two arrows in the figure), but not in the East Asian or West African samples. **B**: Histograms showing the distribution of pigmentation in an African-American and African-Caribbean sample, after adjusting for ancestry for each genotype of the *SLC24A5* rs1426654 SNP. GG homozygotes have higher melanin levels than AG heterozygotes and AA homozygotes (the difference is 9.5 and 7 melanin units, respectively). Source: Lamason et al., 2005. *Science* 310:1782–1786, with permission from AAAS. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley. com.]

threonine, respectively, at amino acid 111 of the protein, show extreme frequency differences between the HapMap European sample and the West African and East Asian samples. In the SNP rs1426654, the ancestral allele, encoding alanine, is the most frequent allele in African, and East Asian populations (93–100%), while the derived allele encoding threonine is nearly fixed (98.7–100%) in European populations (Lamason et al., 2005). In addition to this atypical pattern of differentiation, in the European sample (but not the West African or East Asian samples), there is a dramatic reduction of heterozygosity encompassing a 150 kb region that includes *SLC24A5*, indicating that there has been a recent selective sweep in the ancestral European population (Fig. 9A). The authors then decided to investigate whether SNP rs1426654 plays a role in normal pigmentation variation. With this aim, they tested whether the rs1426654 polymorphism was associated with melanin levels (measured by reflectometry) in two admixed populations: African Americans and African Caribbeans. Lamason et al. (2005) found that individuals with one or two threonine alleles had lighter skin than homozygotes for the ancestral alanine allele (Fig. 9B). It was estimated that *SLC24A5* explains 25–38% of the differences in skin melanin index between populations of European and African ancestry (Lamason et al., 2005). This research emphasizes the usefulness of studies using model organisms to understand the diversity of our species. Additionally, the distribution of the *SLC24A5* polymorphism has important evolutionary implications. The presence of the ancestral alanine allele at very high frequencies and the lack of a selective signature in East Asian populations in the *SLC24A5* gene strongly suggest that the decrease in melanin content took place, at least in part, by different mechanisms in Europe and East Asia. More recently, Norton et al. (2007) characterized the rs1426654 polymorphism in the samples of the Centre d'Etude du Polymorphisme Humain (CEPH) Diversity Panel, which comprise 1059 individuals from 53 populations from different continents (Cann et al., 2002). Figure 10 depicts the distribution of the ancestral alanine and the derived threonine alleles throughout the world. The ancestral alanine allele is present at very high frequencies in sub-Saharan Africa, East Asia, Southeast Asia, the Americas, and Melanesia. In contrast, the derived threonine allele is fixed in European populations (frequency = 100%) and is also present at high frequencies in geographically proximate populations in the Middle East, North Africa, and Pakistan (frequency ranging from 62% to 100%).

Fig. 10. Distribution of the *SLC24A5* A111G polymorphism (rs1426654) in the samples of the CEPH-Diversity panel. The G allele (Ala) is indicated in blue in the online version of the article, and dark gray in the printed version; the A allele (Thr) is indicated in yellow in the online version, and light gray in the printed version. Reproduced from Norton et al., 2007. Genetic evidence for the convergent evolution of light skin in Europeans and East Asians. *Molecular Biology and Evolutuion* 24:710–722 by permission of Oxford University Press. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

MATP (also known as SLC45A2 or AIM1). The MATP (membrane-associated transporter protein) plays an important role in murine pigmentation, and some variants of this gene lead to generalized hypopigmentation of the eyes and fur. In humans, mutations in the *MATP* gene are responsible for oculocutaneous albinism type 4 (OCA4). Recent research indicates that *MATP* shows a pattern of polymorphism that is remarkably similar to what is observed in *SLC24A5*: the leucine variant of the nonsynonymous SNP rs16891982 (encoding leucine or phenylalanine, respectively, at amino acid position 374 of the protein) is mainly restricted to European (and closely related) populations (Nakayama et al., 2002; Graf et al., 2005; Norton et al., 2007), and *MATP* shows a strong signature of selection in European populations. Figure 11 shows the distribution of the rs16891982 polymorphism in the samples of the CEPH Diversity panel, based on Norton et al. data (2007). Two recent studies indicate that *MATP* plays a role in normal pigmentation variation in humans. Graf et al. (2005) have shown that the SNP rs16891982 is strongly associated with dark skin, dark hair and dark eye color in Caucasians. Another nonsynonymous polymorphism (rs26722) was also associated with darker pigmentation. Norton et al. (2007) have also shown that the derived G (leucine) allele of the rs16891982 polymorphism is strongly associated with lighter skin pigmentation in a sample of African Americans ($P < 0.0001$). It was estimated that the ancestral C (phenyalanine) allele increases skin pigmentation by approximately five melanin units and the distribution of pigmentation values per genotype is consistent with an additive mode of action.

OCA2 (also known as the P gene). The human *OCA2* gene is associated with a form of albinism (oculocutaneous albinism type 2). However, evidence is accumulating that this gene also may play a role in normal variation in skin and, more particularly, in iris pigmentation. Akey et al. (2001) described that interaction between the *MC1R* and *P* genes contributes to interindividual variation in skin pigmentation in Tibetans, while Shriver et al. (2003) reported that a variant in the P gene was associated with skin pigmentation in a sample of African Americans and African Caribbeans. *OCA2* also seems to have an important role in iris color. In 1996, Eiberg and Mohr found strong indication of linkage of a region on chromosome 15q, where *OCA2* is located, with eye color and attributed this signal to the *OCA2* gene. Zhu et al. (2004) carried out a sib pair linkage analysis in 502 twin families, and most of the variation in eye color was due to a quantitative trait locus (QTL) on chromosome 15q, near *OCA2* (LOD score = 19.2). This result was recently replicated by Posthuma et al. (2006). In 2002, Rebbeck et al. reported that two *OCA2* polymorphisms (Arg305Trp and Arg419Gln) were associated with eye color. Similarly, Frudakis et al. (2003) reported that several SNPs and haplotypes located within the pigmentation candidate genes *OCA2*, *MYO5A*, *TYRP1*, *MATP*, *DCT*, and *TYR* were associated with iris pigmentation. Associations of SNPs located within *OCA2* were by far the most significant of any gene tested, with 13 SNPs showing strong associations. Recently, Duffy et al. (2007) have described a very strong association of three SNPs within intron 1 of the *OCA2* gene (rs7495174 T/C, rs6497268 G/T, and rs11855019 T/C) with blue eye color. These three SNPs are located in a major haplotype block, and the diplotype (combination of haplotypes) TGT/TGT seems to be a major determinant of iris color, with a frequency of more than 90% in subjects with blue or green iris compared to less than 10% in individuals with brown eye color. In summary, the current evidence clearly indicates that *OCA2* is a major determinant of variation in eye color and also plays a role in skin pigmentation variation.

ASIP. As discussed in the previous section, the agouti signaling protein (ASIP) is the antagonist of MC1R, and upon binding to this receptor, ASIP promotes the synthe-

Fig. 11. Distribution of the *MATP* C374G polymorphism (rs16891982) in the samples of the CEPH-Diversity panel. The C allele (Phe) is indicated in red in the online version of the article, dark gray in the printed version; the G allele (Leu) is indicated in yellow in the online version, and light gray in the printed version. Reproduced from Norton et al., 2007. Genetic evidence for the convergent evolution of light skin in Europeans and East Asians. *Molecular Biology and Evolutuion* 24:710–722 by permission of Oxford University Press. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

sis of pheomelanin. A SNP located in the 3′-untranslated region (UTR) of this gene, g.8818A→G, has been associated with pigmentation phenotypes in humans. In particular, it appears that the *ASIP* 8818G allele is significantly associated with dark hair, brown eyes, and dark skin (Kanetsky et al., 2002; Bonilla et al., 2005). Interestingly, a recent study by Voisey et al. (2006) indicates that the level of *ASIP* mRNA is around 12 times lower in melanocyte cell lines carrying the G allele (homozygotes GG or heterozygotes AG) than in cell lines with the AA genotype. Lower levels of the agouti protein would result in decreased antagonism of α-MSH binding to MC1R and therefore increased production of eumelanin.

TYR. Recent research indicates that the tyrosinase gene (*TYR*), which encodes a key enzyme in melanogenesis and is responsible for one of the two most common types of albinism (oculocutaneous albinism type 1, OCA1), is also involved in normal pigmentation variation. Shriver et al. (2003) described that a nonsynonymous *TYR* polymorphism (rs1042602) was associated with skin pigmentation (measured by reflectometry) in a sample of African Americans and African Caribbeans.

Detecting signatures of selection in pigmentation candidate genes: A promising strategy to identify genes involved in normal pigmentation variation

I would like to close this section by reviewing a promising new strategy for identifying genes involved in normal pigmentation variation using tests to detect signatures of selection in pigmentation candidate genes. The idea behind this approach is that pigmentation candidate genes showing evidence of selection are, or have been in the past, important from the functional point of view, and can potentially explain the variation observed in this phenotype. Indeed, the example of the *SLC24A5* gene clearly shows that this approach has promise to uncover genes involved in pigmentation variation. The action of natural selection leaves a "signature" in the genome, distorting the patterns of genetic variation with respect to neutral expectations. For example, when directional selection has been active in a genomic region, increasing the frequency of an advantageous mutation and linked neutral sites (e.g., a selective sweep), there will be a decrease in nucleotide diversity, an excess of rare variants and an excess of linkage disequilibrium with respect to what is expected under a neutral model (Fay and Wu, 2000; Przeworski, 2002). Numerous tests have been proposed to detect positive selection using data on genetic variation within a species. Some tests evaluate the departure of the allele frequency distribution with respect to neutral expectations (Tajima's D, Fu and Li's D and F, Fay and Wu's H). Other tests evaluate the decrease in heterozygosity (natural log ratio of heterozygosities, LnRH), or the presence of atypical levels of population differentiation (FST; Locus-Specific Branch Length test; LSBL; Informativeness of Assignment index, In). Finally, another group of tests evaluates the decay of linkage disequilibrium (Extented Haplotype Homozygosity test, EHH; iHS test; Linkage Disequilibrium Decay test, LDD). For further information about these tests, refer to Biswas and Akey (2006), McEvoy et al. (2006), and Harris and Meyer (2006). However, the identification of loci that have been the target of selection (directional or balancing) is not straightforward because demographic factors can mimic the patterns of variation produced by selection. As an example, an excess of rare variants with respect to neutral expectations can be due to selective sweeps or population expansions. Conversely, an excess of intermediate-frequency alleles can be due to balancing selection or population bottlenecks (Akey et al., 2004; Williamson et al., 2005). There are two main strategies to solve these potential problems. The first strategy is to incorporate demographic history in the statistical models (Toomajian et al., 2003; Williamson et al., 2005). The difficulty with this approach is that demographic history can be very

TABLE I. List of pigmentation candidate genes showing signatures of natural selection, using different tests[a]

Gene	Test	Population	Reference[b]
***SLC24A5* (Solute carrier family 24, member 5)**	**LSBL, TD, lnRH, FST, EHH, iHS**	**Eur**	**1–4**
***MATP* (*SLC45A2*, Solute carrier family 45, member 2)**	**LSBL, TD, lnRH, FST, In, EHH**	**Eur**	**2–5**
***OCA2* (*P* gene)**	**LSBL, FST, In, EHH**	**Eur, Eas**	**2–5**
DCT (Dopachrome tautomerase)	LSBL, FST, In, EHH	Eas, Afr	2–5
TYRP1 (Tyrosinase-related Protein 1)	LSBL, FST, In, EHH, iHS,	Eur, Afr	1–5
LYST (Lysosomal Trafficking Regulator)	LSBL, TD, lnRH, FST, EHH	Eas, Afr	2,3
MITF (Microphthalmia-associated transcription factor)	LSBL, TD, lnRH, FST, EHH	Eur, Eas, Afr	2,4
KITLG (KIT ligand)	TD, In, EHH	Eur, Eas	2,5
ADAM17 (ADAM metallopeptidase domain 17)	LSBL, TD, lnRH, FST	Eas	2,3
ADAMTS20 (ADAM metallopeptidase with thrombospondin type 1 motif)	LSBL, TD, lnRH, FST	Eas	2,3

[a] Polymorphisms within the genes *SLC24A5*, *MATP*, and *OCA2* (in boldface) have been associated with normal pigmentation variation in previous studies.
[b] 1, Voight et al. (2006); 2, McEvoy et al. (2006); 3, Izagirre et al. (2006); 4, Myles et al. (2007); 5, Lao et al. (2007).
Abbreviations: LSBL, Locus-Specific Branch Length test; TD, Tajima's D test; ln RH, natural logarithm of the Ratio Of Heterozygosities test; FST, FST test; In, Informativeness of Assignment test; EHH, Extended Haplotype Homozygosity test; his, iHS test.

complex (and therefore difficult to model), and it is generally unknown. The second strategy is to compare the patterns of genetic variation of one region with those found in the rest of the genome (Biswas and Akey, 2006). The underlying assumption of this approach is that demographic history will affect the entire genome, while selection will have an effect on specific loci. This method also has potential problems and under certain demographic and selective scenarios, the false discovery rate can be quite large (Kelley et al., 2006; Teshima et al. 2006). However, a recent simulation study indicates that this is a reliable approach to identify genes that have been the target of natural selection (Kelley et al., 2006). In fact, there have been several recent genome-wide scans for positive selection based on the Perlegen (Hinds et al., 2005) and HapMap data (http://www.hapmap.org) that have identified hundreds of genes showing evidence of positive selection, and in spite of the different methods used to detect selection signatures, there is considerable overlap between the studies (Carlson et al., 2005; The International HapMap Consortium, 2005; Voight et al., 2006; Wang et al., 2006; Kelley et al., 2006). Of particular relevance for this review are four recent studies that specifically applied outlier approaches in order to identify pigmentation candidate genes showing evidence of positive selection in samples of East Asian, European, and West African ancestry (Izagirre et al., 2006; McEvoy et al., 2006; Lao et al., 2007; Myles et al., 2007). These studies used a variety of tests to identify signatures of selection, including the Tajima's D- (TD), FST-, LSBL-, In-, LnRH-, and EHH-based tests. Several pigmentation candidate genes had exceptionally unusual patterns of variation, and in many cases the results were consistent using different tests. Some pigmentation candidate genes were also identified as outliers in a previous genome-wide scan published by Voight et al. (2006), using the iHS test. Table 1 shows the list of pigmentation genes for which the results were significant in two or more studies, using different types of tests (for a full list of genes, refer to Izagirre et al., 2006; McEvoy et al., 2006; Myles et al., 2007; and Lao et al., 2007). The fact that these genes are outliers using tests based on different characteristics of the data (allele frequency distribution, heterozygosity, genetic differentiation, and decay of linkage disequilibrium) strongly indicates that they have been the target of positive selection. There are 10 genes in the list, which can be classified in the following categories: 1) genes involved in melanogenesis (*SLC24A5*, *MATP*, *OCA2*, *DCT*, and *TYRP1*); 2) genes involved in melanosome construction/protein routing (*LYST*); and 3) genes involved in melanocyte development and differentiation (*MITF*, *KITLG*, *ADAM17*, and *ADAMTS20*). Previous association studies have indicated that 3 of these 10 genes (*SLC24A5*, *MATP*, and *OCA2*, labeled in boldface in Table 1) play a role in pigmentation variation. One gene shows signatures of selection in the three samples analyzed (*MITF*), five genes in only two populations (*OCA2* and *KITLG* in the East Asian and European sample, *TYRP1* and *DCT* in the European and West African sample, and *LYST* in the West African and East Asian sample), and four genes show signatures of selection in only one sample (*SLC24A5* and *MATP* in the European sample and *ADAM17* and *ADAMTS20* in the East Asian sample). It is important to emphasize that these results have to be interpreted with caution: although the evidence of positive selection appears to be strong, some of these genes have been implicated in biological processes other than pigmentation and it is possible that the selection signatures identified in these genes may be the result of positive selection related to other gene functions. For example, the *ADAM17* gene has been implicated in many processes involved in cell–cell and cell–matrix interactions, including fertilization, muscle development, and neurogenesis. It is also possible that some of these results are just false positives (although this possibility is minimized by the inclusion in the list of genes that are outliers for at least two tests based on different summary statistics). In order to confirm the role of these genes in normal pigmentation variation, genotype/phenotype association studies and/or direct functional studies will be required. To my knowledge, this research has not yet been completed for 7 of the 10 genes (*DCT*, *TYRP1*, *LYST*, *MITF*, *KITLG*, *ADAM17*, and *ADAMTS20*). However, these studies focusing on the identification of potential signatures of selection offer great promise to identify new genes involved in normal population variation. The preliminary data point to a complex picture where positive selection has been acting at different genome locations, and for some genes only in certain population groups. It is interesting to note that although some genes show signatures of selection in more than one population, in some cases there is evidence indicating that these selective events were independent. For example, the gene *OCA2* shows signatures of selection in European and East Asian populations, but the core haplotypes showing evidence of extended homo-

zygosity are different in each population (Lao et al., 2007). Similarly, the two haplotypes showing evidence of selection at the *DCT* gene in East Asians and West Africans are different (Lao et al., 2007). These results highlight the complex evolutionary history of skin pigmentation in human populations, which we are just beginning to understand. A tentative evolutionary model for skin pigmentation pertaining to the three populations (East Asian, European, and West African) that have been studied using these outlier approaches has been recently proposed by McEvoy et al. (2006).

IMPLICATIONS OF PIGMENTATION FOR PUBLIC HEALTH

In a previous section, I summarized the main evolutionary hypotheses that have been put forward to explain the distribution of skin pigmentation in human populations. Although there is still some debate regarding the selective factors involved, it is generally accepted that the strong association between latitude and pigmentation in humans is primarily the result of the action of natural selection promoting the adaptation of human populations to local environmental conditions (in particular UVR incidence), a process that likely took hundreds of generations. However, as a consequence of recent human migrations, many individuals are now living in geographic regions with different environmental conditions than those in which their populations evolved. Because of the key role that pigmentation plays in photoprotection and vitamin D synthesis, these recent migrations have important implications for public health: fair-skinned individuals are at higher risk of several types of skin cancer, particularly in regions with high UVR incidence, and dark-skinned individuals living in high latitude regions are at higher risk for diseases caused by deficient or insufficient vitamin D levels. These issues are further discussed below.

Fair pigmentation and increased risk of skin cancer

Most of the harmful effects of sunlight on the skin (e.g., erythema and DNA damage) arise from exposure to the UV wavelength (Rees, 2004). Melanins are an efficient natural sunblock, particularly in the UV spectrum, so it is not surprising that the risk of developing skin cancer can vary by as much as 100-fold, and is strongly related to skin color (Rees and Flanagan, 1999). The incidences of skin cancer and skin cancer mortality correlate strongly with latitude, with both decreasing further away from the equator (Mielke et al., 2006). Furthermore, skin cancer incidence is higher in fair-skinned populations than dark-skinned populations at the same latitude. Nowhere is this more evident than in Australia, the country with the highest incidence of skin cancer in the world. This applies to both nonmelanoma skin cancer (basal cell carcinoma and squamous cell carcinoma) and malignant melanoma. The incidence of these three types of skin cancer is around 10 times higher in Australia (particularly the northern regions such as Queensland and the Northern Territory) than in Northern Europe (Diepgen and Mahler, 2002; Staples et al., 2006). It has been estimated that one in two Australians will develop some form of skin cancer in his (or her) lifetime (http://www.cancercouncil.com.au/). However, skin cancer disproportionately affects fair-skinned individuals and the Australian aboriginals have a much lower incidence of skin cancers (Sturm, 2002). A recent study has indicated that cutaneous melanin density at the upper inner arm, measured quantitatively by spectrophotometry, is a strong predictor of skin cancer risk, particularly in men (Dwyer et al., 2002). Similarly, skin cancer risk is strongly associated with skin type; individuals with skin type I or II (who burn easily and do not tan) have higher risk than individuals with other skin types (Armstrong and Kricker, 1995). The gene *MC1R* seems to be a key link in this relationship. As discussed in the section devoted to the genetic basis of pigmentation, several *MC1R* variants present in polymorphic frequencies (>1%) in European populations show a substantial reduction in MC1R activity and are associated with red hair and fair skin. Individuals with these *MC1R* variants have poor tanning ability and are prone to burn and to freckle, which are well known risk factors for nonmelanoma skin cancers and malignant melanoma (Sturm, 2002). It is not surprising, then, that the major "red-hair" alleles Arg151Cys, Arg160Trp, and Asp294His significantly increase the risk of developing melanoma (having one allele increases the risk twofold, and having two alleles fourfold) (Sturm, 2002). Similarly, these variants also increase the risk for basal cell carcinoma, squamous cell carcinoma, and solar keratosis (odds ratio approximately 3, Sturm, 2002). The role of MC1R polymorphisms in skin cancer risk is also supported by recent research indicating that melanocytes expressing the variants with decreased MC1R function are more sensitive to the cytotoxic effects of UVR (Scott et al., 2002). Overall, there is very strong evidence indicating that the fair skin typical of northern latitudes (e.g., Northern Europe) is a significant risk factor for different types of skin cancer, particularly in regions with high UVR incidence. The incidence of skin cancers has been increasing dramatically for several decades and has become an important public health concern not only in Australia, but also in Europe and North America (Diepgen and Mahler, 2002; Tucker and Goldstein, 2003). Consequently, numerous prevention programs have been established to lessen the impact of this disease (Saraiya et al., 2004; Stanton et al., 2004).

Dark pigmentation and increased risk of vitamin D insufficiency and deficiency

*S*ynthesis of vitamin D via exposure of the skin to the sun is the most abundant source of vitamin D for most people (Holick, 2003, 2005). UVB radiation (280–320 nm) penetrates the epidermis and photolyzes 7-dehydrocholesterol (7-DHC) to previtamin D_3, which then undergoes thermal isomerization to form vitamin D_3 (Webb, 2006; Chen et al., 2007). Vitamin D_3 enters the circulation bound to the vitamin D-binding protein (DBP), and undergoes two obligate hydroxylations, the first in the liver to 25-hydroxyvitamin D_3 [25(OH)D_3], and the second in the kidney to its active form, 1,25-dihydroxyvitamin D_3 [1,25(OH)D_3] (Holick, 2005). Given the properties of melanin as a natural UVR filter, it is not surprising that the amount of melanin is inversely related to vitamin D production in the skin. According to Holick et al. (2003), people with dark skin (e.g., skin phototype V/VI) require at least 10 times more exposure to sunlight than those with light skin (e.g., skin phototype II/III) to produce the same amount of vitamin D in their skin. Similarly, a recent report by Chen et al. (2007) has demon-

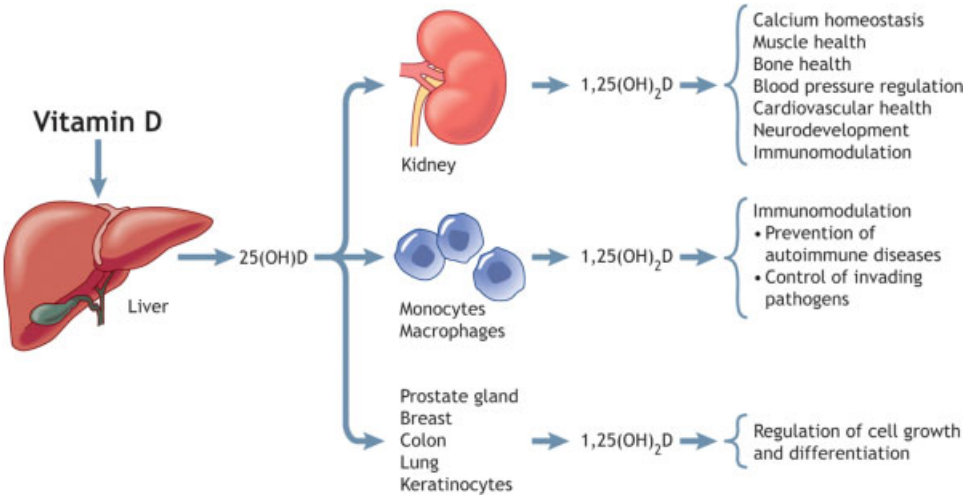

Fig. 12. Organs and tissues capable of synthesis of the active form of vitamin D, $1,25(OH_2)D_3$, showing its multiple physiological roles. Reproduced from Hollis and Wagner, 2006. Nutritional vitamin D status during pregnancy: reasons for concern—reprinted from, *CMAJ* 174(9): 1287–1290 by permission of the publisher. ©2006 Canadian Medical Association. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley. com.]

strated that the conversion of epidermal 7-DHC to previtamin D_3 is 5–10 times more efficient in skin phototype II than in skin phototype V. Therefore, vitamin D synthesis can be compromised by large amounts of melanin, particularly under conditions of limited UVR exposure. Such is the case in high-latitude regions, where there is not enough UVR to synthesize vitamin D for a substantial period of the year (see Fig. 6). Jablonski and Chaplin (2000) did a systematic analysis in which they described the geographic distribution of the potential for vitamin D synthesis. At latitudes around 40° north (Boston), there is insufficient UVB radiation for vitamin D synthesis from November to early March, while at latitudes farther north, like Edmonton, Canada (52° north), this "vitamin D winter" extends from mid-October to mid-March (Webb et al., 1988). Recent research indicates that there is a surprisingly high prevalence of vitamin D insufficiency (typically defined as serum levels of 25-hydroxyvitamin D [25(OH)D] lower than 40–50 nmol/l) in high-latitude countries, even among light-skinned individuals (Lamberg-Allardt et al., 2001; Vieth et al., 2001; Rucker et al., 2002; Tangpricha et al., 2002; Rejnmark et al., 2004). However, the prevalence of vitamin D insufficiency is higher in population groups with higher average levels of melanin. A recent review indicates that in the United States, at equivalent latitudes (25–34.5°N), the prevalence of vitamin D insufficiency is highest in African Americans (52–76%), intermediate in Hispanics (18–50%), and lowest in European Americans (8–31%) (Calvo and Whiting, 2003). Scragg et al. (1995) also reported that in New Zealand (latitude 40°S), Pacific Islanders and Maories had significantly lower concentrations of 25(OH)D than individuals of European ancestry, after controlling for age, sex, and time of the year. The majority of these studies have not controlled for vitamin D intake, which is known to vary significantly among ethnic groups (Calvo et al., 2005). However, a study published by Harris and Dawson-Hughes (1998) reported that in Boston (latitude 40° north), plasma 25(OH)D levels were substantially lower in African American women than in European American women throughout the year, even after adjusting for body weight and vitamin D intake. Unfortunately, none of these studies has evaluated directly the effect of skin pigmentation, measured quantitatively using spectrometry, on vitamin D levels. Therefore, there is very limited information on how melanin levels influence the serum concentration of vitamin D metabolites, after controlling for relevant covariates, such as vitamin D intake (from diet and supplements) and UV exposure.

The current epidemic of vitamin D insufficiency is of public health relevance not only because of the well-known effect of vitamin D on bone metabolism, but also its significant role in protection against numerous chronic conditions. Recent research indicates that many tissues other than the liver and kidney have vitamin D receptors, and are capable of locally synthesizing the active vitamin D metabolite, $1,25(OH)_2D_3$, from the precursor 25(OH)D (Peterlik and Cross, 2005; Hollis and Wagner, 2006). This extrarenal pathway is critical to understanding the effects of vitamin D on immunomodulation and regulation of cell growth and development (see Fig. 12). Adequate vitamin D levels are important not only for protection against rickets, osteomalacia, or osteoporosis, but also for protection against several types of cancer (e.g., breast, colon, prostate cancer), autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis), cardiovascular disease and microbial infections (e.g., tuberculosis) (for recent reviews, see Holick, 2004; Peterlik and Cross, 2005; and Giovannucci, 2005). According to most vitamin D experts, desirable 25(OH)D serum concentration is $\geq$75 nmol/l, and the current vitamin D supplementation recommendations in Canada and the United States (200 international units or IU/day up to age 50 years, 400 IU/day for age 50–70 years, and 600 IU/day over age 70 years) are insufficient for optimal health, so there is urgent need to re-evaluate these recommendations (Vieth et al., 2007). A recent randomized clinical trial (Lappe et al., 2007) has shown that treatment of postmenopausal women with calcium plus 1,100 IU of vitamin D_3 per day dramatically decreases cancer risk compared to women in the placebo control group (RR: 0.402, $P = 0.01$). This and other studies have motivated a recent announcement by the Canadian Cancer Society, recommending that adults living in Canada should consider taking 1,000 IU/day of vitamin D supplementation during the fall and the winter, and adults at higher risk of having lower vitamin D levels (people who are older, those with dark skin, those who do not go outside very often, and/or people who wear clothing that covers most of their skin) should consider taking 1,000 IU/day all year around.

Because of the important autocrine and endocrine functions of vitamin D, ensuring optimal vitamin D lev-

els has become an important public health issue. More research is needed to increase our understanding of how factors such as age, diet, skin pigmentation, geographic location, and intensity of the sun affect the production of vitamin D and to better define the optimal amount of vitamin D supplementation required to prevent health problems in individuals of diverse ancestry.

CONCLUSIONS AND INSIGHTS: WHY STUDY OF PIGMENTATION IS IMPORTANT

I have summarized our current understanding of the evolution and the genetic basis of pigmentation in our species. Pigmentation shows a remarkable diversity in human populations, and in this sense, it is an atypical trait; numerous genetic studies have indicated that the average proportion of genetic variation due to differences among major continental groups is just 10–15% of the total genetic variation. In contrast, skin pigmentation shows large differences among continental populations. The reasons for this discrepancy can be traced back primarily to the strong influence of natural selection, which has shaped the distribution of pigmentation according to a clear latitudinal gradient. It is therefore erroneous to assume that the large population differences observed for skin pigmentation can be extrapolated to most other human traits. In fact, traits that have been strongly shaped by natural selection are notoriously unsuitable to decipher population relationships. Classifications based on traits subject to strong natural selection reflect the *underlying environmental factors*, instead of population history. In this particular case, a classification derived from skin pigmentation mainly captures population differences in UVR exposure, and shows very poor concordance with classifications based on neutral markers, which are the proper tools to use when mapping human history. It is therefore unfortunate that pigmentation has been an omnipresent trait in "racial" classifications that fail to capture or explain the diversity of the human species. However, the study of the genes underlying human pigmentation diversity brings to the forefront the mosaic nature of human genetic variation. Our genome is composed of a myriad of segments with different patterns of variation and evolutionary histories. Due to the recent origin of our species, most of these segments show small differences among human populations, but some genomic regions, particularly those that have been under the influence of natural selection, depart considerably from the average picture of the human genome and show large differences among populations. These genomic regions probably played a key role in the process of adaptation of human populations to different environments and may also explain some of the population differences that have been described in disease risk or drug metabolism. Therefore, in the same way that it is erroneous to extrapolate the patterns of variation observed in superficial traits such as pigmentation to the rest of the genome, it is misleading to suggest, based on the "average" genomic picture, that variation among human populations is irrelevant. In my view, it is paradoxical that, well into the 21st century and into the post-human genome project era, we know so little about the genetic basis of eye, hair, and skin pigmentation. There are many reasons why elucidation of the genetics and evolutionary history of pigmentation is important. 1) Pigmentation is a trait that should be used as an example of how misleading simplistic interpretations of human variation can be, and to educate the general public on the importance of studying variation using an *evolutionary*, rather than a typological, approach. 2) Pigmentation can be very useful to understand the genetic architecture of complex traits. The pigmentation of unexposed areas of the skin (constitutive pigmentation) is relatively unaffected by environmental influences during an individual's lifetime, when compared with other complex traits such as diabetes or blood pressure, and this provides a unique opportunity to study gene–gene interactions without the effect of environmental confounders. 3) Pigmentation is of relevance from a public health perspective, because of its critical role in photoprotection and vitamin D synthesis. Our understanding of the genes involved in normal pigmentation variation in human populations has increased substantially in the last 5 years, and recent studies have identified several pigmentation candidate genes that show strong signatures of natural selection and therefore, may be functionally important in pigmentation. At the current pace of discovery, the next decade will clarify many of the remaining gaps in our knowledge of the genetic architecture and evolutionary history of this fascinating trait.

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