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## *Brief Communications*

### *Skin Reflectance in the Han Chinese and Tibetan Populations*

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*Abstract* Genetic and environmental factors are involved in the determination of skin pigmentation in humans. With the recent development of statistical and genetic tools in mapping complex traits in humans, it is becoming feasible to utilize such methods in identifying genes involved in skin pigmentation. Furthermore, the use of new portable reflectance spectroscopy instruments such as the Photovolt ColorWalk colorimeter allows researchers to measure skin reflectance of a large number of subjects with ease and accuracy. We used a new portable instrument (Photovolt ColorWalk) to study the skin reflectance of 372 Han Chinese and 274 Tibetan individuals to establish background reflectance measurements of unexposed skin of the inner upper arm in these two populations. In addition, we explored the effect of various factors such as age and gender on skin reflectance.

Numerous factors (e.g., environmental, physiological, pathological, and genetic) are involved in the determination of skin color, which constitutes a continuous spectrum of colors ranging from white to black (Harrison and Owen 1964). The contribution of genetic components is significant, given that skin pigmentation varies between populations with different ethnic backgrounds (Harrison 1973). The heritability of skin color is estimated to be 63%–72% (Harrison and Owen 1964), and there may be between two and six major additive genes involved in the determination of skin pigmentation (Davenport and Danielson 1913; Gates 1949; Herskovits 1926, 1930; Stern 1953, 1970).

The reflectance spectroscopy of skin is an objective way to measure skin pigmentation. Such measurement can be used as a phenotype or, more precisely, a quantitative trait in genetic studies. It is hypothesized that only a handful of

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genes are involved in skin and hair pigmentation in humans and animals (Ollmann et al. 1998; Smith et al. 1998; Miller et al. 1997; Vage et al. 1997; Marklund et al. 1996; Barsh 1996). Genetic analyses may eventually lead to the discovery of these genes and the molecular pathways in which they participate. A detailed understanding of the components of the pigmentary system may shed light on the etiology of skin diseases involving the alteration of skin pigmentation such as vitiligo. Moreover, the pigmentation process in skin and/or hair has also been associated with other complex quantitative traits such as obesity (Comuzzie et al. 1997; Seeley et al. 1997; Krude et al. 1998; Jordan and Jackson 1998; Ichii-Jones et al. 1998).

Over the past 50 years, skin pigmentation levels have been objectively studied in anthropology using primarily two lines of instruments for reflectance spectroscopy: E.E.L., manufactured by Evans Electro Selenium Company (Halstead, Essex, England) and Photovolt, manufactured by UMM Electronics (Indianapolis, IN). An abundance of data has been recorded in the literature on pigmentation levels reported using these filter-based reflectometers. However, they are no longer being manufactured and have been replaced by smaller and more accurate devices such as the Photovolt ColorWalk colorimeter. Therefore, it is important to study the distribution of skin reflectance measurements using the new generation of instruments in the populations of interest. To this end, the purpose of this study was to provide background reflectance measurements of unexposed skin of the inner upper arm in the Han Chinese and Tibetan populations using the Photovolt ColorWalk, a new portable instrument.

## Materials and Methods

The Photovolt ColorWalk colorimeter (Photovolt, UMM Electronics, Indianapolis, IN) was used to measure the skin pigmentation of the Han Chinese and Tibetan populations. The ColorWalk is a handheld tristimulus colorimeter, which uses a linear photodiode array to measure the intensity of particular wavelengths of light. Tristimulus colorimetry was developed as a means of objectively representing a particular 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. These filters have been designed to simulate the absorbance spectrum of human eye pigments. Color parameters are then defined by the amount of and differences among the reflectance levels of these three filters. The most commonly used color parameters are the Commission International d'Eclairage  $L^*a^*b$  (CIELab) measurements established in 1976. In the CIELab color system, any color can be represented by three variables that can be plotted in a three-dimensional space:  $L^*$ , the lightness-darkness axis;  $a^*$ , the red-green axis; and  $b^*$ , the blue-yellow axis (Fullerton et al. 1996). Tristimulus colorimeters like the ColorWalk and the commonly used Mi-

nolta Chroma Meter 20 and 300 series machines (Minolta Co., Osaka, Japan) can usually report color values for a number of other color systems as well.

Prior to being measured, subjects were asked several questions including their name, ethnicity, date and place of birth, places of birth of their parents and four grandparents. The Han Chinese is the largest ethnic group (93.6% of the total population) currently residing in the political boundary of the People's Republic of China. Subjects of Han origin were undergraduate and graduate students 18 to 43 years of age currently enrolled in Fudan University, Shanghai. Of the 372 individuals, 293 were male and 79 female; they came from 29 of the 31 provinces in China. Subjects from Tibet consisted of 274 students 12 to 19 years of age at the Shanghai Tibetan School; 126 were male and 148 female. All subjects were measured from September to November 1998. Measurements were taken at the unexposed area of the inner upper arm using the ColorWalk. The instrument was calibrated using the white and black calibration standards supplied by the manufacturer.

## Results

**Correlation of Three Measurements  $L^*$ ,  $a^*$ , and  $b^*$ .** Correlations of the skin reflectance measurements for the Han Chinese samples and for the Tibetan samples are presented in Table 1. All of the correlations presented in Table 1 are statistically significant ( $p = 0.0001$ ).  $L^*$  is negatively correlated with both  $a^*$  and  $b^*$ , while  $a^*$  and  $b^*$  are positively correlated with each other. The correlation coefficient between  $L^*$  and  $a^*$  is greater than those between  $L^*$  and  $b^*$  and between  $a^*$  and  $b^*$ .

**Test of Normality of Skin Reflectance.** We also performed a normality test for skin reflectance with and without a logarithm transformation using the Anderson-Darling normality test, since this data will be used in future genetic studies. In most cases, skin reflectance measures in the population for each gender can be fitted reasonably well by a normal distribution with only two exceptions ( $L^*$  in Tibetan females,  $p = 0.001$ ; and  $b^*$  in Tibetan males,  $p = 0.001$ ). Normality of the skin reflectance with logarithm transformation seems to be slightly worse than that without the transformation (data not shown). It is important to note that al-

**Table 1.** Correlations of the Three Measurements  $L^*$ ,  $a^*$ , and  $b^*$  in the Han Chinese and Tibetan Populations

Population	$L^* - a^*$	$L^* - b^*$	$a^* - b^*$
Chinese	-0.711	-0.384	0.292
Tibetan	-0.692	-0.536	0.424

**Table 2.** Comparison of Skin Pigmentation of Males and Females in the Han Chinese and Tibetan Populations

Measurement	Population	Male		Female		Combined		<i>p</i> -value <sup>a</sup>
		Mean	SE	Mean	SE	Mean	SE	
<i>L</i> *	Chinese	64.41	0.16	67.19	0.27	65.00	0.15	0.0001
	Tibetan	56.60	0.24	60.51	0.26	58.71	0.24	0.0001
<i>a</i> *	Chinese	9.00	0.09	8.40	0.14	8.88	0.08	0.0005
	Tibetan	10.24	0.11	9.37	0.11	9.77	0.08	0.0001
<i>b</i> *	Chinese	17.93	0.12	18.49	0.17	18.05	0.10	0.0068
	Tibetan	20.99	0.14	19.80	0.13	20.35	0.10	0.0001

a. Intrapopulation test of male versus female skin reflectances.

though skin reflectance in our sample fits the normal distribution reasonably well, fit should be verified in all independent data sets.

**Skin Reflectance in Han and Tibetan Samples.** Table 2 shows a comparison of skin reflectance between male and female individuals in the Han and Tibetan populations. In both populations, the skin reflectance differences between male and female individuals are significant for all three measures, *L*\*, *a*\*, and *b*\*. Overall, males tend to be relatively darker than females in both populations as measured by *L*\*. This observation confirmed earlier studies that also showed a significant difference of skin pigmentation in other ethnic groups (Greksa 1998). Table 2 also shows that skin reflectance as measured by *L*\*, *a*\*, and *b*\* differs significantly ( $p = 0.0001$ ) between the Han and Tibetan samples for both genders, and that the former tends to be lighter than the latter as reflected by the *L*\* measure. However, since the age ranges of the Han (18–43) and Tibetan (12–19) samples barely overlap, this data should be viewed with caution and verified in subsequent studies.

The relationship between skin reflectance and age was investigated by dividing the Han Chinese into four age groups (18–22, 23–27, 28–32, and 33–43 years of age) and testing the equality of group means using a one-way analysis of variance. None of the three measurements showed a significant relationship with age. The *p* values of the *F* test for *L*\*, *a*\*, and *b*\* are 0.603, 0.112, and 0.327, respectively.

## Conclusions and Discussion

The purpose of this report was to describe skin reflectance in Han and Tibetan populations using a new colorimeter instrument (Photovolt ColorWalk). Since several of the classical reflectance spectroscopy instruments are no longer being manufactured, it is important to generate population data with the currently available instruments. In our study, males tended to be significantly darker than

females in both the Han Chinese and Tibetan samples. Other researchers (e.g., Greksa et al. 1991; Barnicot 1958; Conway and Baker 1972; Mehrai and Sunderland 1990; Greksa 1998) have reported a similar pattern of sex differences.

Moreover, skin reflectance did not change significantly with the age of the individuals. However, the data did show that  $L^*$  increases and  $a^*$  decreases with age for both males and females, but none of the changes reached statistical significance. This observation is in contrast with that reported by other studies (Greksa et al. 1991; Greksa 1998). In fact, the literature has many examples of complex and contradictory relationships between age and skin reflectance. For example, some researchers have found that upper arm and forehead reflectances were not related to age in males and females (Harrison et al. 1967; Lasker 1954; Mehrai and Sunderland 1990), while others have found that both reflectances increased with age in males and females and that upper arm reflectance measures increased while forehead reflectances decreased in both sexes (Garn et al. 1956; Byard et al. 1984; Rebato et al. 1993; Conway and Baker 1972). A systematic study with a large sample size for each age group is necessary to establish the relationship between skin reflectance and age. Overall, the availability of portable instruments for measuring skin reflectance combined with the development of sophisticated statistical methods (Williams et al. 1999) may allow the genetic architecture of the pigmentation system to be elucidated.

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