

IRIS MELANOCYTE NUMBERS IN ASIAN, AFRICAN AMERICAN, AND CAUCASIAN IRIDES

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ABSTRACT

Purpose: The anatomical basis for iris color has long been a controversial issue in ophthalmology. Recent studies demonstrated that in Caucasians, blue-eyed, gray-eyed, and hazel-eyed individuals have comparable numbers of iris melanocytes. The present investigation was carried out to compare melanocyte numbers in the irides of Asian, African American, and Caucasian brown-eyed individuals.

Methods: Paraffin-embedded sections from 71 brown-colored irides were incubated with rabbit anti-cow antibody against S100a, linked with an FITC conjugate antibody, and counterstained with Evans blue. Cells were counted under a fluorescence microscope and scored as melanocytes or other cells. Cell number, density, and iris area were calculated for each specimen.

Results: Caucasian and African American irides had comparable mean total melanocyte numbers. Asian irides had fewer total melanocytes than African American ($P = .042$) and Caucasian ($P = .001$) irides and smaller total number of cells (ie, melanocytes plus other cells) than African American ($P = .054$) or Caucasian ($P = .009$) irides.

Conclusions: There is a statistically significant smaller mean total melanocyte number and mean total cellularity in Asian irides as compared to Caucasian and African American irides. This difference appears to be due to the combination of smaller iris area and lower melanocyte density in the Asian irides. The possibility exists that this may be a factor in ethnic variations in certain ocular diseases.

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INTRODUCTION

The melanin-containing stromal melanocytes are believed to be the principal factor in determining iris color.¹⁻⁵ But while there is agreement that it is the melanin within the stromal melanocytes that is primarily responsible for the iris color, controversy has long existed as to whether the number of melanocytes, distribution of melanocytes, or the melanin content of individual melanocytes is the determining factor that varies with iris color, or whether these factors are complementary.

Fuchs² believed that the number of melanocytes in the anterior border layer was the principal determinant in

iris color, with darkly pigmented irides having greater numbers of melanocytes. More recently, Dietrich⁴ reached the same conclusion in a light and electron microscopic study of 20 specimens. In contrast to these findings, Wolfrum³ observed that the number of melanocytes in the anterior border layer is relatively constant, irrespective of iris color, but that the amount of pigment within the anterior border layer is greater in darkly pigmented irides. Eagle,⁵ in his American Ophthalmological Society thesis, studied 21 irides, spanning the spectrum of iris color by means of light and electron microscopy. He noted that melanocyte number remains relatively constant but melanosome number and size increase with darkening of the iris.

In a previously reported study, our laboratory systematically examined morphologic differences in iris stroma that contribute to clinically perceptible variations in iris color using immunohistochemical identification of stromal melanocytes and fluorescence microscopy.⁶ Melanocyte numbers, proportion, and density were determined for light-colored (blue), medium-colored (hazel), and dark-colored (brown) irides from 51 Caucasian eyes.

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Bold type indicates **AOSS** member.

No statistically significant difference was observed for the mean total melanocyte number or the mean total cellularity among the three color groups. We concluded that the number of melanocytes, proportion of melanocytes, and iris stromal cellularity are not major contributors to iris color. In a companion electron microscopic study, we found iris color to be associated with the number and area of melanin granules within the superficial iris stroma melanocytes.⁷ The present report extends these previous studies by comparing melanocyte numbers in the irides of Asian, African American, and Caucasian brown-eyed individuals.

MATERIALS AND METHODS

Paraffin-embedded brown eyes that had been donated at autopsy from Asian and African American donors to the Medical Eye Bank of Maryland at Johns Hopkins Hospital were supplied by Dr W. Richard Green and processed for histologic examination. Patient autopsy donations conformed to the Johns Hopkins University Investigative Review Board protocols for use of archival tissues. Control Caucasian brown eyes were sectioned from archival tissue at the University of Wisconsin Eye Pathology Service and were obtained with prior patient consent for use in research studies in accordance with the University of Wisconsin Human Subjects Committee.

Five-micron sections were cut from the paraffin blocks and placed on Plus slides. Sections were rinsed in 0.001 mol/L phosphate-buffered saline (PBS), pH 7.5, for 10 minutes and then underwent digestion with 0.4% pepsin (Sigma Chemical Co, St Louis, Mo) in 0.1 N hydrochloric acid (pH, 2.21) for 15 minutes at 37°C. The slides were blocked in goat serum (Sigma Chemical Co) for 30 minutes at room temperature, incubated with 125 μ L of mouse anti-cow antibody (DAKO, Inc), and conjugated to fluorescein isothiocyanate (anti-rabbit IgG FITC conjugate, Sigma Chemical Co) diluted in PBS 1:1,000 for 1 hour at room temperature. The slides were then rinsed three times in PBS, counterstained with Evans blue (Sigma Chemical Co), and coverslipped.

The slides were examined microscopically at 200 \times magnification by a single reviewer masked to ethnic group using an Olympus BH2 microscope equipped with a fluorescent filter (DAPI/FITC/Texas Red triple band filter set, Chroma Technology Corp, Battleboro, Vt), allowing transmission of three bands of fluorescence between 380 and 680 nm. Under blue filter, the FITC/S100a complex is identified by its greenish yellow color in the cellular cytoplasm of melanocytes, as well as in other cells of neuroectodermal origin, including neurons and pigment epithelium. Nuclei are observed as staining red under fluorescence. Nonneuroectodermal cells, including fibroblasts and clump cells, appear as either red nuclei

without cytoplasmic staining, or as red fibers. These cells are scored as "other cells" for analysis. For each sample, the best section was selected on the basis of cell morphology, stain quality, and stain intensity. All cells in the iris stroma were counted (excluding iris pigment epithelium and muscle cells) as either S100a-fluorescing positive cells or "other cells" (S100a-negative nonfluorescing). Raw cell counts were used for statistical data analysis to determine number of melanocytes and other cells in each specimen.

Adjacent sections of all irides were stained with hematoxylin-eosin and examined using an Olympus BX40-F3 microscope under 40 \times magnification. Digitized images were taken with a camera connected to a computer equipped with a framegrabber board. Using Optimas 6.5 software (Media Cybernetics, Inc), each iris area was traced from the peripupillary border to the iris root and excluding the iris pigment epithelium. The iris cross-sectional area was calculated to square microns (μm^2). Further calculations were done to determine the number of melanocytes per iris area in each specimen. Pairwise comparisons of groups were conducted using the Wilcoxon rank sum test.

RESULTS

Seventy-one iris specimens were analyzed for cell counts and iris area. Results are summarized in Table I. Mean total melanocyte counts for each group were 331 for Asian irides, 439 for African American irides, and 443 for Caucasian irides. The mean total melanocyte cell counts were less in the Asian group when compared to the Caucasian and African American groups and were statistically significant ($P = .001$ and $P = .042$, respectively, Figure 1). Mean cell counts for other cells (fibroblasts and clump cells) were 175 for Asian irides, 208 for African American irides, and 214 for Caucasian irides. These "other" cell counts were thus also less in the Asian group when compared to Caucasian and African American groups. These results are illustrated in Figure 2.

The mean iris areas and mean melanocytes per area (melanocyte density) for each group are also shown in Table I. No significant differences were seen in area measurements of Asian, African American, and Caucasian groups. Note, however, that iris area and melanocyte density are lowest in the Asian group, a combination of factors which may be responsible for the lower total melanocyte count in the Asian group.

DISCUSSION

There are three cell types that contain pigment within the iris: stromal melanocytes, which are derived from the neural crests; iris pigment epithelium, which is derived

Iris Melanocyte Numbers In Asian, African American, and Caucasian Irides

TABLE I: COMPARISON OF MEAN TOTAL NO. OF MELANOCYTES AND OTHER CELLS IN AFRICAN AMERICAN, ASIAN, AND CAUCASIAN BROWN IRIDES

GROUP	NO. MELANOCYTES	NO. OTHER CELLS	TOTAL CELLS
African American	439 ± 147	208 ± 78	647 ± 219
Asian	331 ± 73	178 ± 27	509 ± 81
Caucasian	443 ± 54	225 ± 44	668 ± 86
P VALUE			
African American vs Asian	.042	.22	.054
African American vs Caucasian	.86	.32	.70
Asian vs Caucasian	.001	.017	.001

TABLE II: COMPARISON OF MEAN IRIS AREA AND MEAN MELANOCYTE DENSITY IN AFRICAN AMERICAN, ASIAN, AND CAUCASIAN BROWN IRIDES

GROUP	MEAN IRIS AREA (mm ²)	MEAN ± SEM MELANOCYTE DENSITY, CELLS/mm ²
Asian	0.69 ± 0.16	499 ± 118
African American	0.73 ± 0/21	662 ± 206
Caucasian	0.81 ± 0.52	555 ± 218
P VALUE		
Asian vs Caucasian	.27	.49
Asian vs African American	.55	.19
African American vs Caucasian	.5	.61

Table II: Bold type indicates statistical significance of less than 0.05.

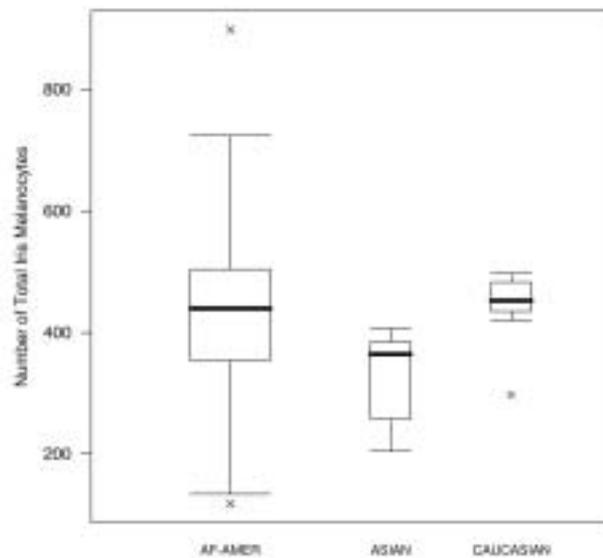


FIGURE 1

Graphical depiction of the melanocyte cell counts in all groups studied.

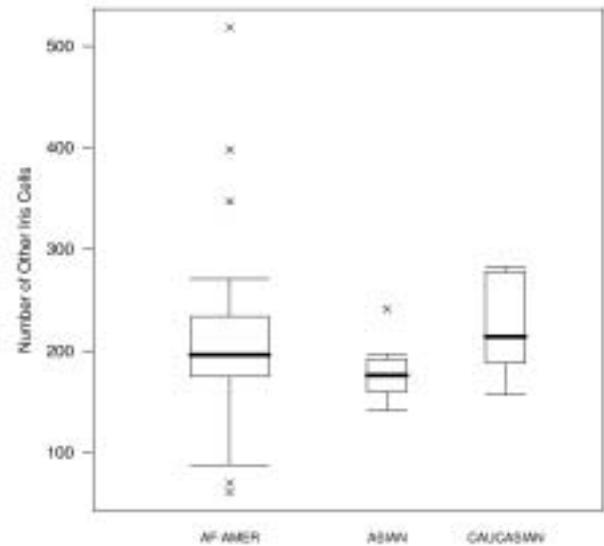


FIGURE 2

Graphical depiction of the other cell counts in all groups studied.

from the neuroectoderm of the optic cup; and clump cells, which are thought primarily to be of histiocytic origin.^{1,8} The pigment epithelium pigmentation does not vary between different iris colors,⁵ and the clump cells are relatively few in number.^{1,8} Consequently, the various

shades of iris color have been mainly attributed to the variability in number and distribution of stromal melanocytes. Our previous studies showed that melanocyte number, total cellularity, and percentage of melanocytes do not vary significantly among the various

Caucasian iris color groups and consequently are not major contributors to iris color.⁶ Rather, iris color appears to be determined by the number and area of melanin granules within superficial iris stromal melanocytes.⁷

In the present study, we compared mean total melanocyte numbers, "other cell numbers," and melanocyte density (number of melanocytes per area of section) in brown Caucasian irides with those in brown African American and Asian irides. The S100a immunostaining technique was used to differentiate between melanocytes and fibroblasts in the iris stroma, based on studies that indicate that S100a protein is found mainly in cells derived from neuroectoderm.^{9,10} The S100a protein contains both the a and b subunits of S100 and is the usual test for S100 protein.¹¹ Accordingly, melanocytes will stain positively for S100a, while fibroblasts and clump cells will not stain. The advantage of using fluorescent microscopy is in the ease of identifying positive and negative immunostaining based on color. All cells in the radial sections were counted, instead of limiting counts to the anterior border layer, to avoid the problem of variation in thickness in the anterior border. Interobserver and intraobserver consistency has been assessed in prior studies, and satisfactory levels of agreement have been seen.⁶

The findings in the fluorescent study indicate that there is no significant difference in the total mean melanocyte numbers found between Caucasian and African American irides. An unexpected finding was that Asian irides had fewer total melanocytes than African American ($P = .005$) and Caucasian ($P = .001$) irides and fewer total number of cells than African American ($P = .054$) or Caucasian ($P = .009$) irides. This appears to be the result of the combination of smaller iris areas and lower melanocyte densities in these eyes.

In the last several decades, associations have been claimed for an increasing number of ocular conditions to differences in iris color. These conditions include glaucoma,¹² age-related macular degeneration,^{13,14} and pigmented tumors of the uvea.^{15,16}

Much attention has also been paid to geo-ethnic variations in ocular disease, and this has stimulated interest in their underlying causality. The finding of fewer melanocytes in Asian irides compared to Caucasian and African American irides raises the possibility that the number of melanocytes may be a factor in ocular disorders with higher or lower incidence in Asian populations. Of the specific eye diseases in which a relationship to uveal melanocytes might be hypothesized, the following entities have a higher incidence in some Asian populations: Vogt-Koyanagi syndrome, Harada's disease, sympathetic ophthalmia, and retinitis pigmentosa, all in Japan.¹⁷

The following diseases, with a possible relationship to uveal pigmentation, have a lower occurrence in some

Asian populations: iris tumors and uveal melanoma in China;¹⁷ iritis, choroiditis, and uveitis, generally in the Singapore Chinese population;¹⁷ and age-related macular degeneration in ethnic Chinese^{17,18} and in Japanese.¹⁹

The prevalence of primary angle-closure glaucoma and primary open-angle glaucoma in Chinese and Asian populations has been studied with varying results.^{17,12,20-23} Ocular disorders that appear to be less common in certain Asian populations as compared to Caucasians include cataract,¹⁷ detached retina,¹⁷ and convergence strabismus.¹⁷ Central serous retinopathy is stated to be common in Japan and Indonesia.¹⁷ It must be stressed that the relationship between numbers of melanocytes and any of these diseases is hypothetical. Demonstration of such a relationship between number of melanocytes and any of these diseases would require prospective data from persons of a variety of ethnic backgrounds using objective systems to document, detect, and classify the presence and severity of lesions with information regarding other risk factors. Finally, it must be noted that the meaning and significance of race and ethnicity in today's world are not well defined and certainly open to question.

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DISCUSSION

DR J. BROOKS CRAWFORD. Back scatter of light by iris stromal collagen fibers is inversely proportional to the fourth power of the wavelength of light; therefore the shorter wavelength blue end of the spectrum gives non-pigmented irides, the sky, and bluebird feathers, none of which have blue pigment, their blue color.¹ Iris melanocytes make up 2/3 of the iris stroma.² The absorptive characteristics of their melanin determine the color of gray, green, hazel and brown irides.¹

Dr Albert and his colleagues previously used the same elegant immunohistochemical techniques they used in this current investigation and ultrastructural studies to show that iris color is not related to the number of melanocytes, but rather to the number and area of melanosomes in the superficial stromal melanocytes.^{2,3}

Dr Eagle in his magnificent AOS thesis devoted 117 pages to iris structure and pigmentation, and reacquainted us with colorful and perhaps forgotten terms such as the mamelons of Gillmaerts and Kleefeld for iris ruff crenations, the greater and lesser rings of Merkel for the ciliary and pupillary zones of the iris and framboisiform cells in iris freckles.⁴ His work agreed with that of Dr Albert and colleagues in finding that brown irides did not contain more melanocytes but did contain more melanin than blue ones.

This current study by Dr Albert and colleagues involved only brown irides. The sample size, 71 irides, was large and therefore gives good statistical power to this work, which can form a basis for future comparisons of iris color related to ocular disease.

Let us remember, though, that the relationship between uveal pigment and various ocular diseases is not necessarily simple. For example oculodermal melanocytosis (nevus of Ota) occurs much more frequently in Asians and African-Americans than in Caucasians, but melanomas arising in the hyperpigmented tissue of oculodermal as well as the more common ocular melanocytosis are far more common in Caucasians.⁵⁻⁷

I have two questions. Could the degree of pupil mydriasis in the Asian irides have been different from that in the African-American and Caucasian irides and thus have accounted for the smaller cross-sectional area and fewer melanocytes in that population? Could this same technique for determining the number and density of melanocytes be applied to the choroid, or is choroidal pigmentation too variable and the amount of tissue too great? Because of the possibility that the depigmented choroidal lesions in Birdshot chorioretinopathy might be due to destruction of choroidal melanocytes, I tried without success to determine the melanocyte density in and adjacent to focal areas of chorioiditis in a recent case that I studied.⁸ Would your technique have been more useful?

Congratulations to Dr Albert and his colleagues for this recent elegant study as well as the two previous ones relating to iris pigmentation. Like Dr Eagle's thesis, they form a sound foundation for future investigations.

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DR GEORGE L. SPAETH. Why are the irides of Asian patients so much more difficult to penetrate with a Neodymium YAG laser? By contrast, one low power shot with the Neodymium YAG laser on a Caucasian will frequently have a huge hole. What's the anatomic basis for that?

DR RALPH C. EAGLE. How did you control for mydriasis or miosis? Did you count all the cells in a single iris leaflet or just a section? It might be interesting if Dr Taylor could supply you some Aborigine irides. That would be interesting to compare with the Asian irises and African irises.

DR MALCOLM R. ING. In Hawaii we get the chance to compare racial distributions of many diseases. We've been impressed with the higher incidence of narrow angle glaucoma in Orientals. We call it creeping angle closure. Since you pointed out that there's a smaller iris area in your cases with Asians, does this imply then a smaller anterior segment and therefore more predisposition to angle closure?

DR DAN B. JONES. My son is a blond, blue-eyed Caucasian and his wife is half-Asian and half-Caucasian, and their little six-month-old daughter has blue eyes. They keep asking me how do we know when the iris color is going to change and are for sure those eyes are going to be blue? My daughter-in-law wants her eyes to be brown.

DR DANIEL M. ALBERT. We did measure and record pupillary size in all of the eyes we received, and there was not a significant difference among the groups. The pupillary size would not affect the total cell numbers counted but could influence area. Can our technique be applied to

the choroid? Yes, I think that this could be done.

Dr Spaeth, it may be that the fewer number of melanocytes in Asian eyes is related to the difficulty in penetrating the iris with the YAG laser, although there may be other factors involved in the differences in collagen and other components of the iris stroma.

Dr Eagle also asked about the size of the pupil. Ralph, we did count all cells extending from the pupillary border to the peripheral iris where it attaches to the ciliary body on a standardized number of sections. If Dr Hugh Taylor or his associates sends us Aborigine irides, we will be happy to study them.

Dr Ing's question on the pathogenesis of narrow angle glaucoma in Asians is much discussed in the literature and may be unresolved. It is my impression that in Asian infants, children, and teenagers, the angle is comparable to Caucasians. Later on in life some develop a narrower iris angle apparently unrelated to the number of melanocytes.

With regard to Dr Jones's questions as to when and how does the iris change color: There are a number of genes exerting an influence on iris color and it is a complex process.