

Eye Color Changes Past Early Childhood

The Louisville Twin Study

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Objective: To determine whether eye color changes after 6 years of age.

Design: Longitudinal data on eye color were obtained from the Louisville Twin Study, Louisville, Ky. Twins ($n=1513$ [individuals]) were assessed at least once and most twins ($n=1386$) were examined on 2 or more occasions. Parents of twins were also examined at the study inception, 128 of whom were assessed again from February 1989 to October 1993.

Main Outcome Measure: Eye color was assessed at each examination by matching the iridial coloration of the subject to 1 of 15 painted glass eye anterior segments, similar to those in artificial eyes, mounted on a circular disk. The spectrum ranged from light blue (1) to dark brown (15).

Results: Among whites ($n=1359$), the eye color of 3.8% to 8.6% of the sample twins became 2 U or more darker

or 2 U or more lighter during 3- to 9-year intervals between 6 years of age and adulthood (>18 years, <24 years). Among identical (monozygotic) twin pairs, there was a high degree of concordance in eye color ($r=0.98$ [$P<.001$]), while in fraternal (dizygotic) twin pairs, the concordance was less pronounced ($r=0.49$) and decreased with age ($r=0.07$). Among the sample of the mothers of twins, 9% had irides that lightened by 2 U or more during the follow-up period.

Conclusion: Most individuals achieve stable eye color by 6 years of age. However, a subpopulation of 10% to 15% of white subjects have changes in eye color throughout adolescence and adulthood in the eye color range that can be expected to reflect changes in iridial melanin content or distribution. These data also suggest that such changes in eye color, or the propensity to such changes, may be genetically determined.

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WHILE IT is generally assumed that a final eye color is achieved during infancy and, in the absence of frank pathology, remains invariant throughout life, data from the Louisville Twin Study, Louisville, Ky demonstrated that eye color changed in 10% to 20% of children between birth and 6 years of age.¹ Eye color concordance was high (90%) among monozygotic twins, but declined among fraternal twins from 80% at 3 months of age to 50% by 6 years of age.

In a cross-sectional, population-based study, the frequencies of blue and brown eyes among males were lower in puberty (age, 11-17 years) than in childhood (age, 4-10 years) and even lower among older males (age, 18-50 years).² The frequency distribution of eye color also varied by age in females, although the pattern was less consis-

tent.² These cross-sectional observations suggest that changes in eye color may occur after early childhood. Longitudinal data are necessary to determine whether changes in iris color occur among all individuals or only in some subsets of susceptible individuals.

Some eyes may retain the capacity to show marked changes in iridial pigmentation during adulthood. Hypopigmentation of the affected iris has been reported to occur after some cases of adult-onset sympathetic degeneration.³ Long-term daily topical application of an ocular hypotensive prostaglandin analogue (latanoprost) has been shown to cause an increase in pigmentation in some adult and elderly individuals. Individuals with peripherally hypochromatic brown-blue, brown-green, or brown-gray irides seemed to be particularly susceptible to change that differentially affected originally hypochromatic areas.⁴

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SUBJECTS AND METHODS

Beginning in 1957, between 20 and 35 pairs of white infant twins were recruited annually from the metropolitan area of Louisville. After 1970, minority (mostly black) and white twin pairs were recruited from families representing the entire socioeconomic range within the metropolitan area, with approximately 8% to 15% of the families representing each decile of a 100-point scale based on parental occupations.⁵

Twins were first assessed at 3 months of age ($n=715$) or as soon thereafter as possible. Follow-up visits occurred at 3-month intervals through 12 months of age, 6-month intervals from 12 to 36 months of age, annually to 9 years of age, and at 12 and 15 years of age, with a final examination between 18 and 24 years of age.

From infancy to 15 years of age, both twins within a pair were measured on the same day by different examiners. After 15 years of age, twins were not always seen on the same day.

During each visit, eye color was assessed by matching the appearance of the eye of the subject (usually the left) to 1 of 15 artificial eye anterior segments mounted on a wheel 13 cm in diameter. Assessments were made next to a window in a single examination room with fluorescent lighting. The subjects were draped with a white cloth to avoid the possible influence of clothing color. The eye color wheel was an adaptation of an earlier eye color scale that used artificial eyes.⁶ Color photography was not used because it was too variable in color reproduction and unstable over time.

Interexaminer reliability was determined several times. Exact agreement for eye color number was greater than 95% for all comparisons ($P<.001$).

The first 6 eye colors (1-6) represented blues of various shades and textures. Since the differences in this range seemed to reflect differences in stromal thickness or textures rather than changes in melanin content or distribution, they were combined into a single category for these analyses.

Lighter to darker shades of gray green and hazel were represented by numbers 7 through 12, and light to dark brown were represented by numbers 13 through 15. In this range (7-15), increasing numbers can be expected to reflect increases in melanin content in the melanocytes of the iris stroma anterior border layer, which is the normal determinant of eye color outside of the blue range.⁷⁻⁹

Eye color was recorded for at least 1 parent during the first visit of the twins and for the other parent in the first year. From February 1989 to October 1993, parents were asked to return for a follow-up visit that included a reassessment of eye color. Data were available for 94 mothers and 34 fathers at 2 examinations an average of 15 years apart. Data for fathers were excluded from analysis owing to the small numbers available.

Zygosity was determined for same-sex twins by blood typing for 22 or more red cell antigens.¹⁰ Change in eye color was defined as a 2-U change in score, which could reflect an increase (darkening) (eg, changing from 6 to 8) or a decrease (lightening) (eg, changing from 10 to 8) in pigmentation.

Data were analyzed using the Statistical Analysis System software package (version 6.09, SAS, Cary, NC). The χ^2 statistic and Mantel-Haenszel test for linear trend were used to evaluate the patterns of change between time points. Hypothesis tests for proportions being significantly greater than 0 used an arc sine transformation or the binomial probability density function.

To determine if eye color changes after 6 years of age, we analyzed additional longitudinal eye color data collected by long-term follow-up assessments of twins participating in the Louisville Twin Study. Longitudinal data on mothers were analyzed to evaluate the potential for change during later adult years.

RESULTS

The mean age at first visit was 0.8 years (9.6 months) with a mode of 0.25 years (3 months) (**Table 1**). On average, twins were seen 9 times during the longitudinal study. The mothers with a follow-up examination were similar in age and eye color to all mothers of twins enrolled in the study.

The **Figure** shows mean eye color of twins by age and race. Because there was little change over time for nonwhite children and the number of nonwhite twins was small, subsequent analyses were limited to white subjects only.

As summarized in **Table 2**, many children demonstrated a darkening of iris color during infancy and early childhood.¹ After 6 years of age, from 7.6% to 16.6% demonstrated a change in eye color (lighter or darker). Among those with a change, about half were lighter and half were darker. During each interval, the percentage changing in

each direction was significantly different from 0 ($P<.05$). There were no differences in these patterns by sex (data not shown).

The frequency of a 2-step eye color change between 6 years of age and adulthood varied by initial eye color (**Table 3**) ($P<.001$). Children with eyes that were hazel to light brown (eye colors No.s 10-12) or darker (eye colors No.s 13-15) at 6 years of age were more likely to experience a decrease in pigmentation by adulthood. An increase in pigmentation was more likely to occur in eyes that were in the gray-green color range (7-9). Twenty percent of the subjects with gray-green eyes at 6 years of age had eyes that were 2 U or more darker by adulthood (Table 3).

We repeated these analyses, randomly selecting a participant from each twin pair, to evaluate the effects of the lack of independence in the full data set. The results were similar but did not reach statistical significance with the greatly reduced sample size (data not shown).

EFFECTS OF ZYGOSITY

The proportions of children experiencing eye color changes did not differ between identical and fraternal twin groups (**Table 4**) ($P=.12$). Within twin pairs, eye color was highly correlated among identical twins

Table 1. Descriptive Characteristics of Individual Participants in the Louisville Twin Study

Twins*	No. (%) of Subjects
Sex	
Male	746 (49.3)
Female	765 (50.6)
Unknown	2 (0.1)
Race	
White	1359 (89.8)
Black	132 (8.7)
Other	20 (1.3)
Unknown	2 (0.1)
Zygosity	
Identical	591 (39.0)
Fraternal	717 (47.0)
Unknown	205 (14.0)
Eye color at 3 mo	
1-6 (blue)	501 (70.0)
7-12 (hazel)	83 (12.0)
13-15 (brown)	131 (18.0)
Eye color at 6 y	
1-6 (blue)	294 (33.0)
7-12 (hazel)	290 (33.0)
13-15 (brown)	299 (34.0)
Eye color at 9 y	
1-6 (blue)	212 (34.0)
7-12 (hazel)	202 (33.0)
13-15 (brown)	204 (33.0)
Eye color at 15 y	
1-6 (blue)	158 (31.0)
7-12 (hazel)	202 (40.0)
13-15 (brown)	144 (29.0)
Eye color at adulthood	
1-6 (blue)	67 (34.0)
7-12 (hazel)	98 (50.0)
13-15 (brown)	31 (16.0)

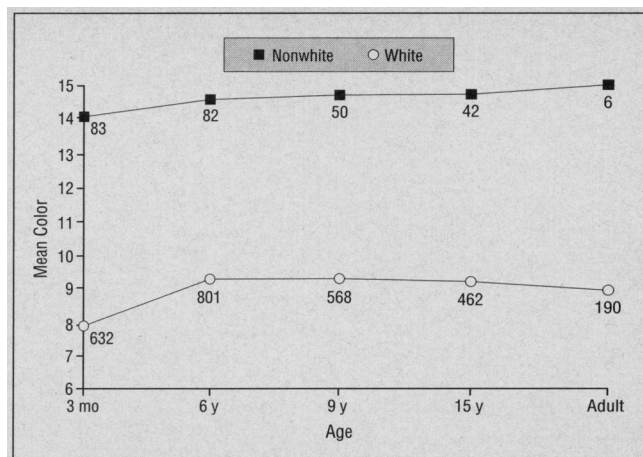
Mother†	All Mothers, No. (%)	Mothers With Follow-up, No. (%)
Race		
White	587 (84.0)	89 (95.0)
Black	63 (9.0)	5 (5.0)
Unknown	48 (7.0)	0 (0)
Eye color at initial visit		
1-6 (blue)	157 (29.0)	30 (32.0)
7-12 (hazel)	199 (37.0)	35 (37.0)
13-15 (brown)	185 (34.0)	29 (31.0)

*For total number of visits, $n=1513$ (mean [SD], 9.0 [4.5]; mode, 13; range, 1-16); for age at first assessment (years), $n=1513$ (mean [SD], 0.8 [1.0]; mode, 0.25; range, 0.25-8).

†Age at birth of twins (years) for all mothers, $n=690$ (mean [SD], 27.1 [5.5]; range, 14-42); for mothers with follow-up, $n=94$ (mean [SD], 27.8 [5.5]; range, 17-42). Age of mothers at follow-up (years), $n=94$ (mean [SD], 44.3 [9.5]; range, 23-69).

at 6 years of age ($r=0.98$ [$P<.001$]) and at adulthood ($r=0.95$ [$P<.001$]). Among fraternal twins, eye color was significantly correlated at 6 years of age ($r=0.49$ [$P<.001$]) but not at adulthood ($r=0.07$ [$P=.67$]).

Identical twins also showed greater concordance of eye color changes by age than fraternal twins, except for the 6- to 9-year age group (**Table 5**). In Table 5, the "change" was considered to be the same in both twins if the eyes of both children stayed the same, both got lighter, or both got darker. The change was "different" if the eyes of one twin stayed the same



Mean iris color by age and race.

Table 2. Changes in Iris Color in Individuals by Age*

Age	No. of Subjects	Same, %	Darker, %	Lighter, %
3 mo-6 y	402	56.0	43.3	0.7
6-9 y	502	92.4	3.8	3.8
9-15 y	332	86.4	6.9	6.6
15 y-adult	149	84.6	8.1	7.4
6 y-adult	187	83.4	8.6	8.0

*White subjects only.

Table 3. Changes in Iris Color From 6 Years of Age to Adult by Baseline Iris Color*

Change	Baseline Iris Color			
	Blues, % (n=77)	Grays/Greens, % (n=40)	Hazels/Light Browns, % (n=33)	Browns, % (n=37)
Lighter	0.0	7.5	18.2	16.2
Same†	90.9	72.5	78.8	83.8
Darker	9.1	20.0	3.0	0.0

*White subjects only. χ^2 test, $P<.01$; Mantel-Haenszel test for linear trend, $P<.001$.

†Same indicates no change.

color while the eyes of the other twin changed in either direction or if the eyes changed in opposite directions.

PARENTS

Paired data from 2 examinations an average of 16.1 years apart were available for 89 white mothers. Overall, 9% of the mothers demonstrated a decrease in eye color during these years while 2.2% darkened. The proportion of eyes getting lighter and the proportion of eyes getting darker differed significantly from 0 ($P<.001$ and $P=.03$, respectively). Mothers with gray-green eyes at baseline were more likely to have a lighter eye color at follow-up (**Table 6**). Nine of the 10 mothers with changes in eye color were younger than 30 years at baseline.

Table 4. Changes in Iris Color in Individuals From 6 Years of Age to Adult by Zygosity*

Result	Identical, % (n=104)	Fraternal, % (n=83)	Overall, % (n=187)
Lighter	8.7	7.2	8.0
Same†	86.5	79.5	83.4
Darker	4.8	13.3	8.6

*White subjects only.

†Same indicates no change.

Table 5. Changes in Iris Color in Twin Pairs by Zygosity*

Age	Identical Twins			Fraternal Twins		
	No. of Subjects	Same,† %	Different, %	No. of Subjects	Same, %	Different, %
3 mo-6y†	101	92.1	7.9	94	62.8	37.2
6-9 y	128	89.1	10.9	123	87.8	12.2
9-15 y†	87	88.5	11.5	79	74.7	25.3
15 y-adult†	40	85.0	15.0	27	59.3	40.7
6 y-adult	48	83.3	16.7	37	73.0	27.0

*White subjects only.

†Same indicates both twins changed in the same direction or both had no change; different, the pattern of change differed between twins.

‡ χ^2 Test ($P < .05$).

COMMENT

Most individuals had stable eye color after early childhood. However, there was a subpopulation of white subjects with eye color changes past childhood. Approximately 17% of twins (Table 3) and 11% of mothers (Table 6) experienced a change in eye color of 2 U or more. These findings are consistent with the cross-sectional article by Burkhardt.² Thus, eye color, and, hence, iridial pigmentation, seems to change in some individuals during later years.

These changes seem to be genetically determined, as indicated by the high concordance of eye color for identical twin pairs and the low degree of concordance for fraternal twin pairs. Furthermore, fraternal twin concordance decreased with age. The pronounced effect of age on concordance among the fraternal, but not identical, twin pairs suggests that in some people, iridial melanocytes may retain receptors, or some other mechanism, to respond to changes in neurohumoral or other influences.

Among twins older than 6 years, the percentage with increasing pigmentation was roughly equal to the percentage with decreasing pigmentation throughout the first 2 decades of life. However, among mothers, the preponderant change was toward lighter irides, suggesting a loss of iridial melanin content. This is in contrast with the first 6 years of life, in which the preponderant change is toward darker irides, presumably reflecting an increase in melanin content. Whether this discrepancy reflects true age-specific differences or the influence of age-related changes in pupil size on color intensity and misclassification, or is the result of

Table 6. Changes in Iris Color in Mothers From Initial Visit to Second Visit By Baseline Iris Color*

Change	Baseline Iris Color			
	Blues, % (n=30)	Grays/Greens, % (n=22)	Hazels/Light Browns, % (n=13)	Brown, % (n=24)
Lighter	0.0	22.7	7.7	8.3
Same†	93.3	77.3	92.3	91.7
Darker	6.7	0.0	0.0	0.0

*White subjects only. χ^2 Test, $P = .07$; Mantel-Haenszel test for linear trend, $P = .19$.

†Same indicates no change.

unmeasured effects of response bias owing to incomplete follow-up in twins and mothers, is not known. Further studies need to evaluate this pattern; a recent article suggests that age-related macular degeneration, a leading cause of blindness in older adults, may be associated with the lightening of eye color during aging.¹¹

The mechanisms that control these changes in iris color remain unknown. Eagle⁷ found virtually identical cell densities in the anterior border layer of irides obtained from blue, tan, or brown eyes. However, the melanocytes from tan or brown irides contained larger and greater numbers of melanosomes than those from blue irides. These observations have recently been confirmed using larger samples, immunohistochemical identification of melanocytes, and digital electron microscope image analysis.^{8,9}

Therefore, changes in eye color seem to reflect changes in the melanin content of iridial melanocytes and changes in melanin turnover. Early studies on cultured iridial and choroidal melanocytes have suggested that these cells lose their capacity to synthesize melanin by the end of the postnatal developmental period. However, given the right tissue culture conditions, iridial melanocytes can effectively synthesize melanin in vitro.¹² Thus, the question of melanin turnover in iridial melanocytes and its neurohumoral control needs to be reexamined. The possible role of neurohumoral influences on these iridial melanocytes is underscored by accumulating evidence showing an unusually high density of autonomic and sensory nerves containing catecholamines, acetylcholine, and various neuropeptides near the anterior surface of the iris in close association with the melanocytes of this anterior border layer.¹³⁻¹⁵

To our knowledge, this article reports the first longitudinal data with standardized assessments of iris color to demonstrate that a subset of the population may experience pigmentary changes throughout the adult years. Understanding the basic mechanisms involved in the normal control of iridial pigmentation will yield important insights into the regulation of iris color.

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Correction

Mistaken Symbol in Abstract and Errors in Figure Citation and Legend. In the article titled "Clinical and Genetic Analysis of a Family Affected With Dominant Atrophy (OPA1)," published in the January issue of the ARCHIVES (1997;115:95-99), an error occurred in the setting of a Greek letter because of a printer output problem. On page 95, parenthetical datum on the seventh line of the second column of the Abstract should have read ($\theta = 0$), not ($\hat{u} = 0$). The additional errors occurred on page 96 in the second column (line 5) under the boxed section, where Figure 2 citation should have been referred to the left, not right, part of Figure 2 and on page 97, where the phrase at the beginning of the second line of the legend for Figure 2 should have read "square symbol indicates," not "square symbols indicate," and the phrase in the middle of the fourth line should have read "Each closed circle," not "Each closed symbol." The journal apologizes for these errors.