Racial Differences of Pigmentation in the Human Vestibular Organs

Isaac D. Erbele, MD1, Frank R. Lin, MD, PhD2,3, Yuri Agrawal, MD, MPH2, Howard W. Francis, MD, MBA2, John P. Carey, MD2, and Wade W. Chien, MD2

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objectives. Melanin pigmentation is present in the human inner ear. In this study, we quantify the melanin pigmentation in the vestibular system and examine racial differences of vestibular melanin pigmentation using human cadaveric temporal bone specimens.

Study Design. Basic research.

Setting. Laboratory.

Subjects and Methods. Light microscopy was used to examine specimens from 40 left temporal bones from the Johns Hopkins Human Temporal Bone Collection. Color images of (1) ampulla of the horizontal canal, (2) utricular wall, (3) endolymphatic duct, and (4) posterior ampullary nerve as it enters the posterior canal were acquired with a digital camera attached to the microscope and image acquisition software. Acquired images of each anatomic area of interest were processed offline through ImageJ. Melanin content was then compared according to ethnicity, age, sex, and location.

Results. Fifteen African American and 25 Caucasian specimens were analyzed. Mean age was 68.8 years. African American specimens had a significantly greater amount of pigment at all 4 sampled locations as compared with Caucasian specimens (P < .01). Between sexes, there was a statistically significant difference (P < .05) at the posterior ampullary nerve, with males having more than females. Melanin content was not associated with age.

Conclusions. There is greater melanin pigmentation within the vestibular system of African Americans than in Caucasians, similar to what has been described in the cochlea. Racial differences in vestibular physiologic function have been observed in the literature and may be explained by differences in melanin pigmentation.

Keywords
vestibule, semicircular canal, utricle, endolymphatic duct, temporal bone, histology, melanin, race, Caucasian, African American

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A lfonso Corti first recognized pigment within the inner ear in 1851 in cows and sheep, but still little is known about its role.1 For the cochlea, there is mounting evidence that skin pigmentation influences noise-induced hearing loss and presbycusis, with darker-skinned individuals having an advantage over lighter-skinned individuals.2–7 Less is known about the role of race and melanin within the vestibular system. Previous studies examining vestibular melanin have found it closely associated with dark cells in areas of increased metabolic activity.8–12 Specifically, melanin is found at increased concentrations in the utricle sac, the dark cell region around the ampullae of the semicircular canals, and the endolymphatic duct (ED).12–16 We examine these areas and compare melanin content based on race, sex, and age. Attention was focused on melanin content of Caucasian and African American specimens, as previous studies found melanin more frequently in the vestibular organs of African Americans than in Caucasians.13,14,16,17 These previous studies examined melanin in the vestibular system qualitatively, but to our knowledge, this is the first attempt to quantify melanin concentration in human vestibular organs.

1Department of Otolaryngology–Head and Neck Surgery, Walter Reed National Military Medical Center, Bethesda, Maryland, USA
2Department of Otolaryngology–Head and Neck Surgery, Johns Hopkins School of Medicine, Baltimore, Maryland, USA
3Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health; Johns Hopkins Center on Aging and Health, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

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Corresponding Author:
Wade Chien, MD, Department of Otolaryngology–Head and Neck Surgery, Johns Hopkins School of Medicine, 601 N Caroline St, Baltimore, MD 21205, USA.
Email: wade.chien@nih.gov
Methods

This study was exempted by our institutional review board.

Temporal Bone Specimens

Human temporal bone specimens were obtained from the Johns Hopkins Human Temporal Bone Collection (Nager Collection). These bones were collected between 1940 and 1988, and demographic information (sex, age, and race) was available from the sample set. They were selected from the same 46 temporal bones as in the Sun et al study, to allow for future comparison with their data set.7 Exclusion criteria were lack of demographic information, presence of other pathology besides presbycusis on the slides, or nonstandard preparation of the specimens, preventing consistent cross-analysis; 40 specimens met these criteria. Temporal bone specimens were prepared in standard fashion, and the methods are more thoroughly described elsewhere.18,19 To discuss briefly, fresh specimens were fixed in formalin, decalcified and dehydrated, and then embedded in celluloid. The specimens were sectioned in the vertical plane at a slice thickness between 20 and 25 μm. Every 10th slide was mounted on a glass slide and stained with hematoxylin and eosin.

Image Analysis

Four locations within the vestibular system were selected according to the presence of melanin and ease of creating standardized images from slides oriented in the vertical plane. The locations are as follows: the ED as it enters the vestibule, the posterior semicircular canal (PSCC) as the posterior ampullary nerve enters, the ampulla of the superior semicircular canal (SSCC) with portions of the superior vestibular nerve, and the utricular wall (UW) directly across from the utricle (Figure 1). The light microscope was used to examine each temporal bone specimen at 10× magnification. White-balanced color images of the visible spectrum were acquired with a digital camera attached to the microscope and image acquisition software (ProgRes; Jenoptik, Jena, Germany). The demographic information was blinded prior, and the images were then analyzed.

Images were then processed and analyzed with ImageJ (Bethesda, Maryland). Pigmentation content in each region was identified through color thresholds. The total pigmentation surface area was then calculated via a particle analysis routine in ImageJ. Conversion to square micrometers was performed with calibrated scale bars. Melanin was expressed as area of melanin staining per high-powered field (HPF). Total image size for each HPF was 1398 × 1053 μm. Further discussion of this method can be found in Sun et al, where it was used to identify melanin in the cochlea.7

Statistical Analysis

After the area of melanin staining per HPF for each area of interest was attained, the data were unblinded. Statistical analysis was performed with Microsoft Excel 2007 (Redmond, Washington). The area of melanin staining per HPF was analyzed by sex and ethnicity at all points of interest. The area of melanin staining per HPF of ED, PSCC, SSCC, and UW was averaged and expressed as average vestibular melanin (AVM). A normal distribution was not

Figure 1. Representative slides of endolymphatic duct, posterior semicircular canal and posterior ampullary nerve, superior semicircular canal and superior ampullary nerve, and utricular wall, divided by race. Melanin appears red in these images. H&E, hematoxylin and eosin.
presumed; Mann-Whitney U tests were used to compare melanin concentrations with 2-tailed P values. Proportional data were analyzed with a χ² test. Linear regression was performed to compare age with AVM.

Results

From the 46 specimens used in the study by Sun et al, 40 met the inclusion criteria for this study. The demographic information is presented in Table 1. Vestibules of 15 African American and 25 Caucasian individuals were included, and there were 13 women and 27 men. There was no statistically significant difference in age or sex of the Caucasian or African American group.

Melanin staining was encountered throughout the vestibular system, and the highest concentration appeared to be in the dark regions around the ampullae. Melanin was not found within the maculae of the saccule and the utricle. Limited melanin was encountered in the semicircular canals proper. Some melanin was apparent around nerves and blood vessels. However, like what was found by LaFerriere and colleagues, this appeared to be a minor contribution. Melanin staining was identified in every specimen at all 4 locations.

The median quantities of melanin staining detected in the ED, PSCC, SSCC, and UW sections were 659, 1480.5, 604, and 389.5 μm² per HPF, respectively (Figure 2). There was a statistically significant difference between the SSCC and ED, as well as between the SSCC and UW (P < .05). The quantity of melanin staining detected in the ED, PSCC, SSCC, and UW sections of each specimen was averaged and expressed as the AVM; the median AVM across specimens was 954.6 μm².

There was a statistically significant difference between the AVM of the African American group and that of the Caucasian group (P < .0001), with the median area of melanin staining per HPF being 3438 and 382 μm², respectively. There was no statistical significant difference of the AVM between the female and male groups (Figure 3).

There was also a statistically significant difference in the area of melanin staining per HPF in the African American group versus the Caucasian group when the ED (P < .001), PSCC (P < .01), SSCC (P < .0001), and UW (P < .0001) were examined individually (Figure 4). The median melanin per HPF in African Americans versus Caucasians was 1842 vs 198 μm² in the ED, 1548 vs 162 μm² in the PSCC, 3492 vs 640 μm² in the SSCC, and 1768 vs 164 μm² in the UW. There was a statistical significant difference between sexes for area of melanin staining per HPF in the PSCC (P < .05),
which was 336 μm² in females and 1135 μm² in males (Figure 5). This statistical significance is likely due to 3 outliers in the male PSCC data, however. No significant differences were found between the sexes at other vestibular locations.

Linear regression of age versus AVM demonstrated no correlation between age and melanin content of the vestibular structures (R² = 0.02; Figure 6).

Discussion
This study suggests a large and significant difference in melanin content of the vestibular end organs between temporal bone specimens of African American and Caucasian donors. In this series, African Americans had a 9-fold-greater melanin concentration in their vestibular system than their Caucasian counterparts. The finding of an association between vestibular melanin and skin pigmentation is consistent with previous studies in humans and animals.12-14,16,17,20

In contrast to previous human studies of melanin staining in the vestibular system,13,14,16,17 every individual represented in this study had at least some melanin staining in each of the 4 locations examined. In our series, there were some specimens in both racial groups with very little observable melanin, and these might have been recorded as having none in the previous qualitative studies.

Regarding sex, there is a statistically significant difference in the PSCC series. One previous study without statistical analysis did note a sex difference in melanin presence.16 In our study, this difference in the PSCC series most likely represents a statistical anomaly. Due to orientation of the cuts through the vestibular end organs, the images were slightly inconsistent when the posterior ampullary nerve and the ampulla of the posterior semicircular canal were examined. Images were intended to focus on the nerve as it entered the semicircular canal, which occasionally missed the dark cell region and the ampulla. This may have resulted in greater number of outliers in the PSCC series. No statistical significance by sex was identified at the other 3 locations.

No correlation was identified between melanin and age, and previous studies of melanin in the vestibular system do not describe age-related differences outside the lack of presence in fetuses.16 Melanin concentration does appear to be positively correlated with age elsewhere in the inner ear—namely, the stria vascularis.7 The stria vascularis is likely more metabolically active than the portions of the vestibule studied here, which may account for this difference.

The role of melanin in the vestibular system is unknown, although a handful of studies examined its clinical significance. Specifically, these studies examined racial differences in the aging vestibular system21,22 and melanin and its relation to potential inflammatory processes, such as Ménière’s disease and labyrinthitis.13,15,23-27 Additionally, Waardenburg syndrome, a well-known congenital pigment disorder, is known to affect the vestibular system.28-31

Figure 5. Locations within the vestibule by sex. Note the logarithmic scale. ED, endolymphatic duct; F, female; HPF, high-powered field; M, male; PSCC, posterior semicircular canal; SSCC, superior semicircular canal; UW, utricular wall. *P > .05.

Figure 6. Linear regression of age versus average vestibular melanin. HPF, high-powered field.
The studies of race and the aging vestibular system are of particular interest to this discussion. In 2009, Agrawal and colleagues examined the National Health and Nutrition Examination Survey data between 2001 and 2004, when surveillance balance testing was conducted. They did not find a racial difference in loss of balance in that data set. Their group followed this study with more specific vestibular testing—namely, ocular and cervical vestibular evoked myogenic potentials. They found that ocular vestibular evoked myogenic potential amplitudes were greater and negative wave latencies longer in African Americans than in Caucasians, and they suggested that melanin may be protective against age-related declines in utricular function.

The relationship between Ménière’s disease and melanin is less clear. In early epidemiologic studies of Ménière’s disease in sub-Saharan Africa, very few patients meeting diagnostic criteria were identified. Later studies, however, suggest that the incidence is closer to that of Europeans. In the United States, it appears that Ménière’s disease is less common in African Americans than in Caucasians.

Experimental models of endolymphatic hydrops in guinea pigs were found to have melanogenesis and activation of melanocytes, and activation of melanocytes was more common in more heavily pigmented animals. Similarly, 5 of 6 Ménière’s patients in Gussan’s study were found have hyperpigmented EDs and sacs. Gussan also found hyperpigmentation in all 5 of his patients with labyrinthitis, however, and it is possible that hyperpigmentation represents a more general response to inflammation. A more recent study exposed lipopolysaccharides to the vestibules of mice, which resulted in melanogenesis, and the authors theorized that this activation helps to maintain the blood-labyrinth barrier.

In addition to the acquired vestibular pathologies of aging and Ménière’s disease, melanin plays a role in Waardenburg syndrome. Waardenburg is characterized by its stigmata as well as varying degrees of congenital deafness, caused by failure of melanocytes to migrate from the neural crest to their target organ. Waardenburg syndrome as originally defined did not include vestibular dysfunction, despite identifying patients with vestibular disorders in his initial series. Contemporary studies, however, have identified a higher likelihood of abnormal vestibular testing in Waardenburg patients, and histopathologic examination of the temporal bones of patients with type 1 Waardenburg syndrome has found morphologic abnormalities of the utricle, saccule, and ampullae of the semicircular canals. The failure of melanocytes to migrate into the labyrinth may be associated with varying degrees of vestibular dysfunction in Waardenburg patients.

While this is one of very few studies of melanin within the human vestibular system, it has some significant limitations. Notably, lipofuscin—an age-related pigmented by-product that can appear yellow-brown—is difficult to distinguish from melanin. Immunohistochemical staining would have been able to distinguish between melanin and lipofuscin. Unfortunately, the tissue blocks for our specimens were destroyed long ago, and we could not perform this validation step.

One additional weakness of this study is that it does not differentiate types of melanin. Described methods of differentiating types of melanin were beyond the scope of this study. Previous work has demonstrated eumelanin and pheomelanin in the ampullae and utricles of guinea pigs, as well as neuromelanin in the sacculles of chinchillas. Although their role is unknown in the vestibular system, animal models of the cochlea have suggested a protective effect with eumelanin and a toxic effect with pheomelanin. The presence of pheomelanin in the vestibular system may suggest a complicated relationship between the different types of melanin and the vestibular system.

Another limitation of this study is that we do not know the skin melanin concentration of the individuals represented in these studies. These slides are simply indexed as “black” and “white.” Ignoring the cultural constructs of how a person defines oneself as black or white, we do not know the Fitzpatrick scale of the individuals represented.

Conclusion

This study serves as a reference for melanin content in the human vestibular system. Our study demonstrates more melanin in the vestibular system of African American subjects than in Caucasian subjects. This finding is consistent across the ED as it enters the vestibule, the posterior semicircular canal as the posterior ampullary nerve enters, the ampulla of the superior semicircular canal with portions of the superior vestibular nerve, and the utricular wall directly across from the utricle. There is no difference in melanin concentration in these areas based on age, and there does not appear to be a difference with regard to sex, with the possible exception of the posterior semicircular canal. The role of melanin within the vestibular system is unknown, but it may be protective.

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Author Contributions

Isaac D. Erbele, concept/design, acquisition, analysis, interpretation, drafting/revisions, final approval, agreement of accountability; Frank R. Lin, concept/design, acquisition, analysis, interpretation, drafting/revisions, final approval, agreement of accountability; Yuri Agrawal, concept/design, acquisition, analysis, interpretation, drafting/revisions, final approval, agreement of accountability; Howard W. Francis, concept/design, acquisition, analysis, interpretation, drafting/revisions, final approval, agreement of accountability; John P. Carey, concept/design, acquisition, analysis, interpretation, drafting/revisions, final approval, agreement of accountability; Wade W. Chien, concept/design, acquisition, analysis, interpretation, drafting/revisions, final approval, agreement of accountability.

Disclosures

Competing interests: Isaac D. Erbele, New York City Transit Association—consulting fees (eg, advisory boards); Frank R. Lin, Amplifon—speaker/honoraria, including speakers bureau,
symposia, and expert witness; Autifony—consultant/advisory board; Cochlear Ltd—consultant/advisory board; Med El—speaker/honoraria, including speakers bureau, symposia, and expert witness; Pfizer—consultant/advisory board; Howard W. Francis, Med El, Advanced Bionics, Cochlear Corporation—surgical advisor boards; John P. Carey, Otonomy—other research support (including receipt of drugs, supplies, equipment, or other in-kind support); research grant (including principal investigator, collaborator, or consultant and pending grants as well as grants already received).

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