PRIMARY eye care

Researchers from the USA National Eye Institute (NEI) have recently discovered five subpopulations of retinal pigment epithelium (RPE)— a tissue in the retina that is vital to human visual perception.

The RPE layer of tissue, located between the photoreceptors and choroid, nourishes and supports the retina's light-sensing photoreceptors and in this RPE layer distinct differences among the cells have now been identified.

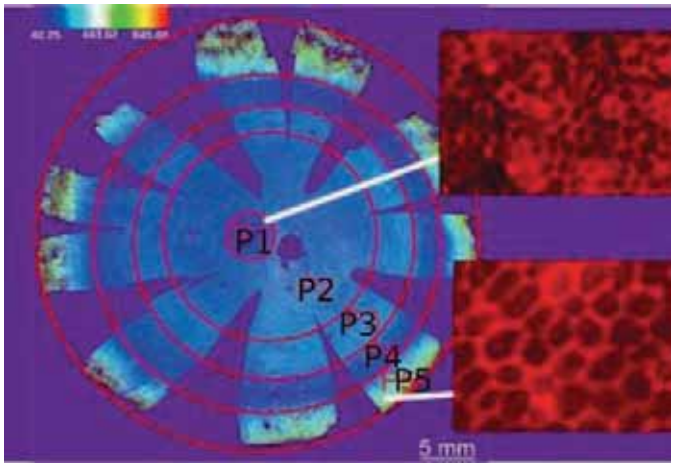
It is known that age and disease can cause metabolic changes in RPE cells that can lead to photoreceptor degeneration. The impact on vision from these RPE changes varies dramatically by severity and where the RPE cells reside within the retina. For example, late-onset retinal degeneration (L-ORD) affects mostly peripheral retina and, therefore, peripheral vision. Age-related macular degeneration (AMD), a leading cause of vision loss, primarily affects RPE cells in the macula, which is crucial for central vision. It is these location factors that lead Bharti and colleagues to design their study to determine if there are different RPE subpopulations that might explain the wide spectrum of retinal disease phenotypes.

Using artificial intelligence, the researchers analyzed images of RPE at single-cell resolution to create a reference map that locates each subpopulation within the eye.

These results provide a first-of-its-kind framework for understanding different RPE cell subpopulations and their vulnerability to retinal diseases, and for developing targeted therapies to treat them.

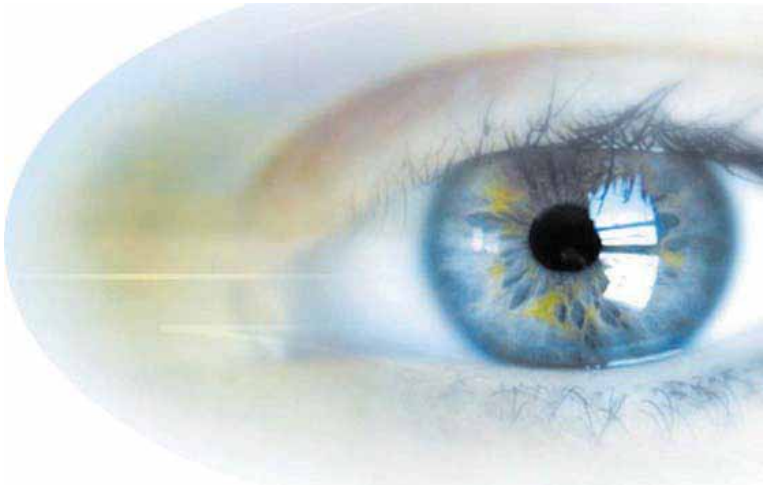
"The findings will help us develop more precise cell and gene therapies for specific degenerative eye diseases," said the study's lead investigator, Kapil Bharti, Ph.D., who directs the NEI Ocular and Stem Cell Translational Research Section.

The team used artificial intelligence (AI) to analyze RPE cell morphometry, the quantitative analysis of form that encompasses size and shape. Morphometric analyses are commonly performed on organisms, and are useful in analyzing their fossil record, the impact of mutations on shape, and developmental changes in form.



Caption: Using artificial intelligence, the researchers analyzed RPE from nine donors and identified five subpopulations of RPE. These populations are along a spectrum in terms of cell area, aspect ratio, hexagonality and number of neighbours. Foveal RPE (P1) required for central vision tend to be perfect hexagons and are tightly packed together. Peripheral RPE (P5) are less perfect hexagons and are more spread out. Credit: Davide Ortolan, Ph.D.

Bharti et. al. trained a computer using fluorescently labelled images of RPE to analyze the entire human RPE monolayer from nine cadaver donors with no history of significant eye disease.



Morphometry features were calculated for each RPE cell – on average, about 2.8 million cells per donor; 47.6 million cells were analyzed in total. The algorithm assessed each cell's area, aspect ratio (width to height), hexagonality, and number of neighbors. Previous studies had suggested that RPE function is tied to the tightness of cellular junctions; the more crowded, the better for indicating cellular health.

Based on morphometry, five distinct RPE cell subpopulations were identified. These were referred to as P1-P5, organized in concentric circles around the fovea, which is the center of the macula and the most light-sensitive region of the retina. Compared to RPE in the periphery, foveal RPE tend to be perfectly hexagonal and more compactly situated, with higher numbers of neighbouring cells.

Unexpectedly, they discovered that the peripheral retina contains a ring of RPE cells (P4) with a cell area very similar to RPE in and around the macula

"The presence of the P4 subpopulation highlights the diversity within retinal periphery, suggesting that there could be functional differences among RPE that we are currently unaware of," said the study's first author, Davide Ortolan, Ph.D. a research fellow in the NEI Ocular and Stem Cell Translational Research Section. "Future studies are needed to help us understand the role of this subpopulation."

Next, the team analyzed RPE from cadavers with AMD. Foveal (P1) RPE tended to be absent due to disease damage, and the differences among cells in the P2-P5 subpopulations were not statistically significant. Overall, the AMD RPE subpopulations tended to be elongated relative to RPE cells not affected by AMD.

To further test the hypothesis that different retinal degenerations affect specific RPE subpopulations, they analyzed ultrawide-field fundus autofluorescence images from patients affected by choroideremia, L-ORD, or a retinal degeneration with no identified molecular cause. While these studies were conducted at a single point in time, they still demonstrated that different RPE subpopulations are vulnerable to different types of retinal degenerative diseases.

"Overall, the results suggest that AI can detect changes of RPE cell morphometry prior to the development of visibly apparent degeneration," said Ortolan.

Age-related morphometric changes also may appear in some RPE subpopulations before they're detectable in others. These finding will help inform future studies using noninvasive imaging technologies, such as adaptive optics, which resolve retinal cells in unprecedented detail and could potentially be used to predict changes in RPE health in living patients.

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References:

Ortolan D, Sharma R, Volkov A, Maminishkis A, Hotaling NA, Huryn LA, Cukras C, Di Marco S, Bisti S, Bharti K. "Single Cell-Resolution Map of Human Retinal Pigment Epithelium Helps Discover Subpopulations with Differential Disease Sensitivity". Published May 6, 2022 in PNAS. https://doi.org/10.1073/pnas.2117553119