Teacher Resource for:
SLC24A5, a Putative Cation Exchanger, Affects Pigmentation in Zebrafish and Humans.

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NOTE: Prior to working through the annotated paper, educators may want to assign the HHMI film *The Biology of Skin Color*, which provides an excellent background for the research presented in the article. An interactive version of the film with embedded pause points and quiz modules is also available.

Student Learning Goals

Connections to the nature of science from the article

- Approaches used to map and test *slc24a5* as the *golden* gene have been around for more than 10 years, but nobody had completed the work.
- Although the effect of the human *SLC24A5* allele on skin pigmentation is large, it does not account for all skin color variation, even together with other known genes.
- This paper required the expertise of many scientists from different fields using different experimental approaches.
- Conservation of certain traits, like pigmentation, in organisms other than humans means these organisms can be used as models to understand human traits.
- Large phenotypic differences can be caused by minor changes in gene sequence, which in turn can change the protein amino acid sequence or the amount of gene expression.
- The study created a testable model for the role of calcium in melanosome function.

The importance of this scientific research

- Clarifies the origin of a common zebrafish mutant that is used to calibrate genetic screens in vertebrate development.
- Identifies a major polymorphism selected for in Europeans that results in lighter skin pigmentation.
- Highlights the merit of collaborative projects such as the human genome project and HapMap project.
- Highlights the relevance of model organisms to the study of human biology.

The actual science involved

- Positional cloning, gene knockdown, and gene rescue experiments to implicate a particular gene as responsible for a trait.
- Microscopy of fluorescently labeled proteins to determine their location in the cell. The location of a protein helps determine its function.
- Comparative genomics: Large-scale sequencing of genomes from geographically isolated populations can help identify regions that are subject to a certain selective pressure.
- Importance of human test subjects in quantitative analyses: quantifying skin pigmentation by measuring reflectance, and determining the frequency of genotypes by genotyping admixed populations.

Connect to Learning Standards:

The National Academies of Science, Engineering, and Medicine Core Ideas of Life Science

- Evolution and its underlying genetic mechanisms of inheritance and variability are key to understanding both the unity and the diversity of life on Earth.

The Common Core English and Language Arts Standards

- ELA-Literacy.RST.11-12.4: Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11 and 12 texts and topics
- ELA-Literacy.RST.11-12.7: Integrate and evaluate multiple sources of information presented in diverse formats and media (*e.g.*, quantitative data, video, multimedia) in order to address a question or solve a problem
- ELA-Literacy.RST.11-12.8: Evaluate the hypothesis, data, analysis, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information.
- ELA-Literacy.RST.11-12.9: Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible

The Common Core Statistics and Probability Standards

- Make inferences and justify conclusions from sample surveys, experiments and observational studies

AP Biology Quantitative Skills

- Looking for trends or associations by determining linear regressions is a key quantitative skill covered on the AP Biology exam.

<u>Summary of the Article for the Teacher:</u>
It is recommended that this not be used by students in place of reading the article.

General Overview:

Human skin color is a highly variable trait. Human skin cells contain the pigment melanin, which gives skin its color. In general, individuals with lighter skin tones have fewer, smaller, and less densely pigmented melanosomes, the melanin-producing organelles, in their skin cells than individuals with darker skin tones.

To better understand the genetic origin of variation in human skin color, Rebecca Lamason and colleagues turned to a model organism: the zebrafish (Danio rerio), which also displays variations in skin color. They identified a gene (called *golden*) that, when mutated, leads to more lightly pigmented, or *golden*, fish. Whereas wild-type zebrafish have numerous dense, round-to-oval melanosomes in their skin cells, the melanosomes of *golden* zebrafish are less numerous, smaller, and less densely pigmented. The scientists searched for an ortholog (a corresponding gene of similar sequence and function) of the *golden* gene in humans. The closest match was a gene called *SLC24A5*. Like the *golden* gene, the *SLC24A5* gene encodes a membrane protein that affects melanosome production.

To determine the gene's role in human skin color, the researchers searched for polymorphisms within the gene. They identified one single-nucleotide polymorphism with two alleles. The G allele, which encodes alanine, is found in most individuals in African, Indigenous American, and East Asian populations (with an allele frequency of 93% to 100%), whereas the A allele, which encodes threonine, is found in European-American populations (frequency of 98.7% to 100%). They then studied two populations of recently mixed ancestry, African-American and African-Caribbean, with a range of skin colors to determine whether allele frequencies correlate with skin pigmentation. Skin pigmentation was measured using reflectometry, which involves measuring the amount of light reflected back by an individual's skin to calculate the melanin index. Individuals with a higher melanin index have darker skin.

Topics Covered:

- model organism genetics
- organelle biology
- gene identification
- model building
- human population genetics
- protein function (ion pumps)

Methods used in the Research:

- electron microscopy of melanin-producing tissue
- postitional cloning to identify mutated gene
- gene knockdown, rescue experiments, and gene expression across tissues
- flourescence microscopy for protein localization within cells
- large-scale human genotyping
- quantification of pigmentation
- data analysis and statistics

Conclusions:

- The loss of *slc24a5* function is responsible for the reduced pigmentation in *golden* zebrafish.
- The *slc24a5* gene has an evolutionarily conserved function in fish and mammals (including humans) where it affects melanin pigmentation.
- The *slc24a5* protein encodes a membrane protein that affects melanosome formation and the production of melanin. It is probably an ion pump that maintains ion homeostasis in melanosome organelles.
- A human *SLC24A5* gene variant that causes lighter skin pigmentation was under evolutionary selection in ancestors of present-day Europeans.
- The variation in human *SLC24A5* explains more of the difference in skin pigmentation between Europeans and Africans than any other gene identified to date.

Areas of Further Study:

- How human *SLC24A5* and other known pigmentation genes work together to produce the diversity of human skin pigmentation.
- The molecular function of the human *SLC24A5* protein in melanosome organelles.
- Other genes that could explain the remaining heritability of skin pigmentation in humans (*SLC24A5* only explains 25%!).
- How skin pigmentation augments physiological processes relevant to human health (for example the production of vitamin D).

Discussion Questions

1. Do you think the researchers started this project to understand human skin pigmentation? Why or why not?

2. How did the authors use the genetic variation of another species to better understand the trait of human skin color?

3. The *slc24a5* gene encodes a membrane protein that affects the transport of cations like sodium and calcium (Na^+ and Ca^{2+}) across the membrane. Why was it important to find out where within the pigment cells of zebrafish the *slc24a5* protein was located?

4. Why did the authors study human populations of mixed ancestry?

5. Based on your background reading, what human ancestry most commonly has the GG genotype and why? What ancestry most commonly has the AA genotype and why?

6. How does the amount of melanin in the skin cells relate to skin color?

7. In Figure 6B, the distributions of skin melanin content for individuals of each genotype overlap. What would you see if only one gene determined skin color? What can this mean in terms of the number of genes that may be involved in skin color?

8. What might be a physiological role or function for the range of skin pigmentation?

9. What do the results of this study tell us about the role of genes in determining skin color?

10. The ancestral G allele of the *SLC24A5* gene, which encodes alanine, is found in most individuals in African, Indigenous American, and East Asian populations, whereas the A allele, which encodes threonine, is found in European-American populations.

11. The researchers found that both of these *SLC24A5* alleles could restore pigmentation in *golden* zebrafish. Because the A allele is associated with lighter skin pigmentation, why do you think the allele could restore pigmentation in zebrafish?

12. Although the human *SLC24A5* gene is only about 20 kb long, the region with extensive loss of heterozygosity in Europeans (Figure 5) is about 150 kb long. What is the significance of this difference in length?

13. What are some examples of other human traits that could be studied in model organisms like zebrafish and mice? Are there any human traits that might be hard to study in these organisms? How could these traits be studied?

Multimedia Resources from HHMI's BioInteractive (www.BioInteractive.org)

Animations

How We Get Our Skin Color (http://www.hhmi.org/biointeractive/how-we-get-our-skin-color). This engaging animation shows how human skin cells produce the pigment melanin and how the type, abundance, and distribution of melanin determines a person's unique skin color.

- Interactive Animation with Quiz: http://www.hhmi.org/biointeractive/how-we-get-our-skin-color-interactive

Polymerase Chain Reaction (PCR) (http://www.hhmi.org/biointeractive/polymerase-chain-reaction). Polymerase chain reaction, or PCR, is a technique for making many copies of a specific DNA sequence. This animation shows how it works.

Related Topics

DNA Transcription. Using information from molecular research, this 3-D animation shows how DNA is translated into RNA, an important step in the process of synthesizing proteins.

- Advanced detail: http://www.hhmi.org/biointeractive/dna-transcription-advanced-detail
- Basic detail: http://www.hhmi.org/biointeractive/dna-transcription-basic-detail

Short Films

The Biology of Skin Color (http://www.hhmi.org/biointeractive/biology-skin-color). In this film, anthropologist Dr. Nina Jablonski walks us through the evidence that the different shades of skin color among human populations arose as adaptations to the intensity of UV radiation in different parts of the world.

- Interactive Video with Quiz: http://www.hhmi.org/biointeractive/skin-color-interactive-video
- Film Guide: http://www.hhmi.org/biointeractive/film-guide-biology-skin-color

Virtual Labs

Bacterial Identification Virtual Lab (http://www.hhmi.org/biointeractive/bacterial-identification-virtual-lab). This virtual lab will familiarize you with the science and techniques used to identify different types of bacteria based on their DNA sequences.

Classroom Activities

Data Point: Genetic Origin of Variation in Human Skin Color (http://www.hhmi.org/biointeractive/genetic-origin-variation-human-skin-color). In this short activity, students interpret and discuss a histogram from the annotated paper

Lamason *et al.* 2014. The accompanying educator guide includes discussion questions on the characteristics of the histogram and what it shows.

Biochemistry and Cell Signaling Pathway of the Mc1r Gene (http://www.hhmi.org/biointeractive/genetic-origin-variation-human-skin-color). An advanced lesson that requires students to analyze partial DNA sequences of the *Mc1r* gene and identify the effects of mutations on the MC1R protein pathway

Human Skin Color: Evidence for Selection (http://www.hhmi.org/biointeractive/human-skin-color-evidence-selection). This case study is based on the short film *The Biology of Skin Color*. Students use real data to propose hypotheses, make predictions, and justify claims with evidence.

Related Topics

Using Genetic Crosses to Analyze a Stickleback Trait (http://www.hhmi.org/biointeractive/using-genetic-crosses-analyze-stickleback-trait). A hands-on activity in which students analyze the results of genetic crosses between stickleback fish with different traits.

Interactive Tutorials ("Click and Learns")

DNA Sequence Assembly (http://www.hhmi.org/biointeractive/dna-sequence-assembly). Learn the principles of how DNA is sequenced and assembled into whole genomes using the Sanger method, shotgun sequencing, or ultra-deep sequencing.

Teacher Guides

Gene Expression (http://www.hhmi.org/biointeractive/teacher-guide-gene-expression). This curriculum guide assists in filtering through the available resources from BioInteractive and HHMI on topics related to gene expression, including RNA structure and function, transcription, RNA processing, translation, and post-translational events.

DNA (http://www.hhmi.org/biointeractive/teacher-guide-dna). This curriculum guide assists in filtering through the available resources from BioInteractive and HHMI on topics related to DNA, including DNA structure and function, DNA replication, damage to DNA, and eukaryotic chromosomal structure.

Image of the Week

Crystals and the Color of Skin (http://www.hhmi.org/biointeractive/crystals-and-color-skin). The panther chameleon alters the arrangement of tiny crystals in its skin to change color.

Coloring the Past (http://www.hhmi.org/biointeractive/coloring-past). An electron micrograph of tightly-packed melanosomes in a 55.4-million-year-old fossil bird feather unearthed in Denmark.

Collections

Biology of Cells (http://www.hhmi.org/biointeractive/biology-cells). Resources for teaching cell biology, including short films, animations, Click & Learn interactives, and posters.

DNA (http://www.hhmi.org/biointeractive/dna-collection). A variety of engaging animations, lecture clips, virtual labs, and other classroom resources teach key concepts related to DNA's structure and function.

Genetics (http://www.hhmi.org/biointeractive/genetics). resources for teaching genetics, including short films, animations, Click & Learn interactives, and classroom activities.

Evolution (http://www.hhmi.org/biointeractive/evolution-collection). Resources for teaching evolution, from short films to Click & Learn interactives, and from lecture series to classroom activities.

Organismal Biology http://www.hhmi.org/biointeractive/organismal-biology Resources related to biology of organisms, including physiology, anatomy, infectious diseases, and developmental biology.

Chemistry of Life http://www.hhmi.org/biointeractive/chemistry-life Resources related to chemistry, biochemistry, and biological macromolecules such as DNA, RNA, proteins, carbohydrates, and lipids.

Introductory Biology http://www.hhmi.org/biointeractive/introductory-biology Multimedia resources that enhance college-level biology instruction. Short films, stunning high-quality animations and data-rich virtual labs impart the thrill of discovery and illuminate the scientific process.