A single DNA tweak leads to blond hair

June 1 2014

Credit: Laura Tiitto/public domain

A single-letter change in the genetic code is enough to generate blond hair in humans, in dramatic contrast to our dark-haired ancestors. A new analysis by Howard Hughes Medical Institute (HHMI) scientists has pinpointed that change, which is common in the genomes of Northern Europeans, and shown how it fine-tunes the regulation of an essential gene.

"This particular genetic variation in humans is associated with blond hair, but it isn't associated with eye color or other pigmentation traits," says David Kingsley, an HHMI investigator at Stanford University who led the study. "The specificity of the switch shows exactly how independent color changes can be encoded to produce specific traits in humans." Kingsley and his colleagues published their findings in the June 1, 2014, issue of the journal *Nature Genetics*.

Kingsley says a handful of genes likely determine hair color in humans, however, the precise molecular basis of the trait remains poorly understood. But Kingsley's discovery of the genetic hair-color switch didn't begin with a deep curiosity about golden locks. It began with fish.

For more than a decade, Kingsley has studied the three-spined stickleback, a small fish whose marine ancestors began to colonize lakes and streams at the end of the last Ice Age. By studying how sticklebacks have adapted to habitats around the world, Kingsley is uncovering evidence of the molecular changes that drive evolution. In 2007, when his team investigated how different populations of the fish had acquired their skin colors, they discovered that changes in the same gene had driven changes in pigmentation in fish found in various lakes and streams throughout the world. They wondered if the same held true not just in the numerous bodies of water in which sticklebacks have evolved, but among other species.

Genomic surveys by other groups had revealed that the gene – Kit ligand – is indeed evolutionarily significant among humans. "The very same gene that we found controlling skin color in fish showed one of the strongest signatures of selection in different human populations around the world," Kingsley says. His team went on to show that in humans, different versions of Kit ligand were associated with differences in skin color.

Furthermore, in both fish and humans, the genetic changes associated with pigmentation differences were distant from the DNA that encodes the Kit ligand protein, in regions of the genome where regulatory elements lie. "It looked like regulatory mutations in both fish and humans were changing pigment," Kingsley says. Kingsley's subsequent stickleback studies have shown that when new traits evolve in different fish populations, changes in regulatory DNA are responsible about 85 percent of the time. Genome-wide association studies have linked many human traits to changes in regulatory DNA, as well. Tracking down specific regulatory elements in the vast expanse of the genome can be challenging, however. "We have to be kind of choosy about which regulatory elements we decide to zoom in on," Kingsley says. "We thought human hair color was at least as interesting as stickleback skin color." So his team focused its efforts on a human pigmentation trait that has long attracted attention in history, art, and popular culture.

Kit ligand encodes a protein that aids the development of pigment-producing cells, so it made sense that changing its activity could affect hair or skin color. But the Kit ligand protein also plays a host of other roles throughout the body, influencing the behavior of blood stem cells, sperm or egg precursors, and neurons in the intestine. Kingsley wanted to know how alterations to the DNA surrounding this essential gene could drive changes in coloration without comprising Kit ligand's other functions.

Catherine Guenther, an HHMI research specialist in Kingsley's lab, began experiments to search for regulatory switches that might specifically control hair color. She snipped out segments of human DNA from the region implicated in previous blond genetic association studies, and linked each piece to a reporter gene that produces a telltale blue color when it is switched on. When she introduced these into mice, she found that one piece of DNA switched on gene activity only in developing hair follicles. "When we found the hair follicle switch, we

could then ask what's different between blonds and brunettes in northern Europe," Kingsley said. Examining the DNA in that regulatory segment, they found a single letter of genetic code that differed between individuals with different hair colors.

Their next step was to test each version's effect on the activity of the Kit ligand gene. Their preliminary experiments, conducted in cultured cells, indicated that placing the gene under the control of the "blond" switch reduced its activity by about 20 percent, as compared to the "brunette" version of the switch. The change seemed slight, but Kingsley and Guenther suspected they had identified the critical point in the DNA sequence.

The scientists next engineered mice with a Kit ligand gene placed under the control of the brunette or the blond hair enhancer. Using technology developed by Liqun Luo, who is also an HHMI investigator at Stanford, they were able to ensure that each gene was inserted in precisely the same way, so that a pair of mice differed only by the single letter in the hair follicle switch—one carrying the ancestral version, the other carrying the blond version.

"Sure enough, when you look at them, that one base pair is enough to lighten the hair color of the animals, even though it is only a 20 percent difference in gene expression," Kingsley says. "This is a good example of how fine-tuned regulatory differences may be to produce different traits. The genetic mechanism that controls blond hair doesn't alter the biology of any other part of the body. It's a good example of a trait that's skin deep—and only skin deep."

Given Kit ligand's range of activities throughout the body, Kingsley says many such regulatory elements are likely scattered throughout the DNA that surrounds the gene. "We think the genome is littered with switches," he says. And like the hair color switch, many of the regulatory elements

that control Kit ligand and other genes may subtly adjust activity. "A little up or a little down next to key genes–rather than on or off–is enough to produce significant differences. The trick is, which switches have changed to produce which traits?

"Despite the challenges, we now clearly have the methods to link traits to particular DNA alterations. I think you will see a lot more of this type of study in the future, leading to a much better understanding of both the molecular basis of human diversity and of the susceptibility or resistance to many common diseases," Kingsley said.

More information: Paper: dx.doi.org/10.1038/ng.2991

Provided by Howard Hughes Medical Institute