Medical Xpress

Natural blood doping and rewriting the textbooks

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Drs. Anders Waage and Anders Sundan, both with the Norwegian University of Science and Technology, deduced the paradoxical effect of a mutation in a Norwegian family, amending a classic tale in genetics. Credit: NTNU

The phrase "rewriting the textbooks" is more than a cliché to me, because that's what I do. I revise each of my books every three years, updating the science.

I love to explain biology through cases and stories, and am disturbed when something changes – that is, when new evidence indicates that facts aren't as they seemed.

Sometimes it's hard for me to give up favorite stories. Worst was the case of Phineas Gage.

Phineas Gage

I used Gage's strange tale to open a nervous system chapter in a few editions of Hole's Human Anatomy and Physiology, up until 2010.

Mr. Gage was 25 on a September day in 1848, when he was working in Vermont, smoothing terrain to lay down railroad tracks. He'd drill holes, fill them with gunpowder, cover them with sand, insert fuses, and then press down with an iron rod called a tamping iron. Boom! The rock would shatter.

But on that fateful day, Gage tamped before his co-worker had sanded, and the whole thing blew up, "slamming the inch-thick, 40-inch-long iron rod straight through Gage's skull, piercing his skull like an arrow shooting through a soft melon," I wrote. A dramatic personality change landed him in the psychology books, demonstrating that specific brain parts control certain qualities. In 1994, his harpooned, famed organ took up residence at the Warren Anatomical Museum at Harvard Medical School, alongside the offending tamping iron.

What was so fascinating about Gage was the supposed changes he underwent after walking away from the accident. The Guardian quotes from a report:

"He is fitful, irreverent, indulging at times in the grossest profanity (which was not previously his custom), manifesting but little deference for his fellows, impatient of restraint of advice when it conflicts with his desires, at times pertinaciously obstinent, yet capricious and vacillating, devising many plans of future operation, which are no sooner arranged than they are abandoned in turn for others appearing more feasible. In this regard, his mind was radically changed, so decidedly that his friends and acquaintances said he was "no longer Gage."

But people began to question the story. The Guardian article points out the missing facts, like not knowing what Gage was like before the accident, and for how long he remained altered. With so many uncertainties, I removed Phineas.

The "Mad King"

I also dropped the story of King George III, aka the "mad king," and the attribution of his wild symptoms to porphyria variegata. The tale was in all editions of my human genetics textbook until the most recent, the twelfth, published last year. Before that, the story threaded through six editions of my intro biology textbook Life. I was sad to see it go.

My genetics textbook had two illustrations of the biochemical pathway that porphyria variegata interrupted in the unstable leader. Here's part of the description:

"At age 50, he first experienced abdominal pain and constipation, followed by weak limbs, fever, a fast pulse, hoarseness, and dark red urine. Next, nervous system signs and symptoms began, including insomnia, headaches, visual problems, restlessness, delirium, convulsions, and stupor. His confused and racing thoughts, combined with actions such as ripping off his wig and running about naked while at the peak of a fever, convinced court observers that the king was mad. Just as Parliament was debating his ability to rule, he recovered."

The king continued to experience these episodes, and finally sunk into a permanent stupor and was dethroned. It can indeed be disturbing when a leader is mentally unbalanced.

In George's case, the red urine was a clue to the underlying blood disorder, described in the *British Medical Journal*, in 1966. Then a report in *The Lancet* in 2005 on analysis of the king's hair revealed high levels

of arsenic, which might have explained the symptoms or exacerbated porphyria. But unfortunately the samples didn't yield any DNA.

With so much uncertainty, I removed the King George story from the 12th edition of my book.

Excess EPO Boosts Blood

In the next edition, I'll need to update the story of Eero Antero Mäntyrantapedia, a famed skier from Finland who won seven medals at three of the four Winter Olympics he competed in from 1960 to 1972. Mäntyranta's prowess came from his body producing too much of the kidney hormone erythropoietin (EPO), which increases the hemoglobin (Hb) level in the blood. Athletes illegally inject EPO – a form of blood doping – because of its hemoglobin-boosting ability. According to the

label of one product, it "supercharges athletic performance."

If the famed Finnish skier was blood doping, he was doing it with more than 30 relatives, for they all had a form of familial erythrocytosis that doesn't cause symptoms. All the relatives were well and many had no idea they were unusual.

Researchers at the University of Helsinki traced the family's mutation, a single DNA base change, five generations back to one person in the 1850s. The trait is dominant, so there aren't carriers.

By 1993, methods had progressed to the point that researchers finally identified the family's mutation, in the EPOR gene, which normally encodes the EPO receptor. The abnormal receptor stays "on" even when the hormone is no longer binding, like a stuck doorbell. That ups the output of red blood cells as well as the level of hemoglobin in the blood.

So when I read a news release in early June, "Family blood mystery solved," and saw the Finnish skier story, I thought I'd have to jettison another favorite genetics story. Maybe like King George, the Finnish skier's endurance wasn't thanks to his genes after all. But it was more complicated than that. It turns out that Mäntyranta's mutation isn't the only way to inherit familial erythrocytosis.

In the The *New England Journal of Medicine* report that the news release announced, a team from Norway and Switzerland described another family with dominantly-inherited erythrocytosis: 10 individuals over four generations make excess EPO. But they have mild symptoms, headaches and dizziness relieved by having blood removed – just like the treatment for the much more common hereditary hemochromatosis that impairs disposal of iron from broken-down hemoglobin. But the second extended family didn't have an EPO receptor mutation like the Finnish skier and his family.

This new chapter began in the late 1970s, when physicians at Namsos Hospital north of Trondheim, Norway, saw a man with unexplained high Hb levels, as did four close relatives. The doctors were puzzled, but published a paper describing the family in 1983.

Then in the early 1990s, Anders Waage, MD, Ph.D., from the Norwegian University of Science and Technology, began seeing the family.

"Normal Hb values are between 12.5 and 17 (grams of hemoglobin per 100 ml of blood), whereas people with this condition have Hb values around 20. These are way over the doping limit," he said in the news release. The Finnish skier's hemoglobin was 22.

By 2008, genomewide mapping had improved to the point that the research team, now larger, could finally zero in on the mutation – also helped by tracking new affected family members. "A limited region of the genome was found to be identical in all affected members of the family. This region contained 215 genes, which were sequenced. To everyone's great surprise, they found a mutation in the EPO gene," Dr. Waage said.

A Paradox

But something didn't make sense. How could a glitch in the EPO gene rev up hormone production, rather than slow it?

An answer came with the availability of CRISPR gene editing, invented in 2012. Gene editing enabled the researchers to recreate the family's mutation in cultured cells, to watch what was going on. And inserting the mutation into liver cells upped EPO output ten-fold! With that clue, the researchers determined that the family's bodies pump out the blood-boosting hormone not only from their kidneys, but from their livers too.

The fact that the family's gene is missing just one DNA base is responsible for the effect on the blood. Any mutation that adds or removes a number of DNA bases that is not 3 or a multiple of 3 "disrupts the reading frame" in which 3 contiguous bases spell out, to the cell, a specific one of the 20 amino acids. It's like a typo that alters word breaks.

Imagine the sentence "I have a feeling we are not in Kansas anymore." Delete two letters – fe – and regroup the words, and it becomes "I have a elingwe ar eno ti nKansa sanymore." Genetic gibberish.

The one missing DNA base in this "frameshift" mutation renders meaningless the EPO gene as it is normally read from a start point. The family members with the mutation shouldn't make any EPO at all, but instead they make too much. How does this happen? Enter the plasticity of the genome, like a vast encyclopedia that can be read from many points.

It turns out that the normal version of the EPO gene is indeed different, but the mutation enables transcription of RNA to begin from a different initiation point in the gene – a little like starting that sentence about Dorothy in Oz with the second word, "have" instead of "I." An alternate messenger RNA is knitted and peels off the gene, leaves the nucleus, and the cell translates the information and strings together the amino acids to form EPO – more efficiently, in fact, than normal. Geneticists call this a "gain-of-function" mutation, because something new happens.

Alas, most people who inherit the ability to make more hemoglobin aren't Olympic athletes, although they're generally pretty healthy. But blood doping does, temporarily, increase endurance. Maybe that explains the bottles at the health food store.

I wonder what other stories I'll have to revise.