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EVOLUTION

The Huntington's PARADOX

The gene that causes a devastating neurodegenerative disease may also have been critical in the evolution of our species

By Chiara Zuccato and Elena Cattaneo

IN BRIEF

Huntington's disease, a serious genetic disorder affecting the brain, is caused by a mutation in which some genetic code letters of a person's DNA are repeated too many times.

Studies have now reconstructed the evolutionary history of the affected gene, which first appeared more than a billion years ago and still can be found in most species today.

The disease may be an unfortunate by-product of an evolutionary process. Expanding numbers of the code letters in the gene appear to assist in nervous system development.

Repeated code letters may increase over many generations. An individual who has a certain number of repeats develops the uncontrolled movements of Huntington's.

Illustration by Andrea Ucini

FOR 15 YEARS BRITISH INSURERS HAVE AGREED NOT to use information about a prospective policyholder's genes to determine eligibility for certain life insurance policies. The moratorium has one critical exception. An underwriter can take into account when writing some policies whether a person carries the gene for the malady once known as chronic hereditary chorea and now simply Huntington's disease.

Becoming aware of a positive gene test lets insurers know that, in the continuing absence of any intervention, the applicant's cause of death will likely be Huntington's—knowledge that comes with far greater certainty than other factors they typically consider, such as smoking, drinking or riding a motorcycle. Someone with the errant gene may begin to experience mood shifts and memory disturbances right in the prime of life, usually between the ages of 30 and 50, although these changes can occur later. Then the symptoms will worsen, expanding to include uncontrollable movements and spasms and an unstable walk often described as a halting "dance." Little by little, the body will lose all its functions, slowing down until complete immobility sets in, and the person finally succumbs to the disease.

Researchers have understood for many years that an aberration of the gene known as *huntingtin* causes the condition. All humans carry *huntingtin* because it is important in the development of the nervous system before birth. But the gene differs slightly from person to person, and these differences explain why some become ill and others stay healthy.

One section of the gene contains a triplet of nucleotides, or "DNA code letters"—namely, C-A-G—that repeats multiple times in a row. In people who remain healthy, the number of CAG triplets ranges between eight and 35. If the number is higher, an individual will eventually sicken with the disease, named after George Huntington (1850–1916), the physician who first described it. One bad gene copy (of the two *huntingtin* genes that each of us inherits from our parents) is sufficient to cause the disorder, and each child of an affected parent has a high—50 percent—chance of carrying the gene. As a consequence of this inheritance pattern, one in 10,000 individuals in Europe and America is afflicted.

Investigators have also known that Huntington's symptoms result from the death of neurons in the corpus striatum and the cortex, areas of the brain that control body movements and higher cognitive functions. Consequently, a good deal of the research into the disorder aims to identify how the high-repeat versions of the gene cause such damage and to develop drugs that can halt the relentless progression of symptoms.

Our laboratory, along with many others in various countries, devotes much energy to these efforts. Several years ago in the course of that research, a number of us became intrigued as well by the broader question of why harmful versions of the gene persist generation after generation instead of being weeded out by natural selection. We wondered whether a biological game of brinkmanship is at work. Is there some survival or reproductive benefit to our species in having large numbers of genetic repeats but perhaps not too many? People suffering from the disease also ask this question; they understand that the answer will probably not cure anyone, yet they still want to know.

Recently investigations into this puzzle have led to intriguing insights into the gene's role in the development of the nervous system in humans and other organisms. It turns out that an increasing number of CAG repeats appears to promote the functioning of neurons, as long as the rise does not surpass the disease threshold. In this sense, Huntington's may be less of a genetic disorder than an unfortunate by-product of a brain-shaping evolutionary process gone awry. A genetic change that might make us "smarter" appears to lead to tragic consequences if pushed too far. Therein lies the paradox of Huntington's.

IN THE BEGINNING

DETECTIVE WORK THAT LED to our current understanding of the part the gene plays in the evolution of the nervous system required researchers to peer back more than a billion years to the advent of the ancestors of both humans and a multicellular amoeba called *Dictyostelium discoideum*. These early life-forms lived between the Paleoproterozoic and the Mesoproterozoic eras and were the first to carry the gene, though in a form that was slightly different from the human version.

Descendants of *D. discoideum* amoebas can still be found living in soil and decaying leaves on the forest floor and feeding on bacteria. They allowed Miguel Andrade-Navarro, then at the Max Delbrück Center for Molecular Medicine in Berlin, and his group to search complex databases and find the gene in the amoeba in 2009. Andrade-Navarro and his collaborators discovered that one of the ways the amoeba's Huntington's gene (a less formal name for *huntingtin*) differs from the human kind is that it possesses no CAG triplets. Nevertheless, the gene

appears to take on a critical part in one stage of the organism's life by letting single-celled amoebae join up with others to form a multicellular entity called a pseudoplasmodium.

This conglomeration of amoebae fends for itself better than a single amoeba when food is scarce or environmental conditions are otherwise harsh. In 2011 Michael Myre and James Gusella of Massachusetts General Hospital reported that the gene regulates a number of vital cellular processes, including the transition of *Dictyostelium* to its multicellular stage. Individual cells lacking the Huntington's gene move about with difficulty and are unable to aggregate normally with other cells. The gene thus appears key to cells that need to "socialize" with one another to survive.

In fact, the gene has many functions. A team at Johns Hopkins University has discovered that it controls when amoebae reproduce and regulates their response to stimuli from their surroundings that impel them to move toward food. In our lab we found that the *Dictyostelium* version of the gene protects mammalian cells from stimuli that trigger cell death.

The amoeba preceded the division of the tree of life into its two branches more than 550 million years ago: the protostomes, which include insects, crustaceans, mollusks, and the deuterostomes, which led to the first vertebrates—the fishes, birds, amphibians, reptiles, mammals, primates and modern human beings. Only the deuterostomes went on to accumulate CAG triplets at the place in the gene where the disease-causing mutation in humans is found.

As we discovered in 2008, the Huntington's gene starts to acquire CAG triplets in a category of basal deuterostomes called echinoderms (such as the sea urchin *Strongylocentrotus purpuratus*). Working with a group of scientists at our university in Milan who specialize in computing techniques for biology, we deciphered the DNA sequence of the sea urchin gene, identifying two CAG triplets in the initial part of the gene.

In this creature, the DNA sequence still differs from that of the human gene. Despite the presence of a primitive nervous system in sea urchins, the gene is present mainly in nonneural tissues. Its absence suggests that early on in evolution, the gene and its two CAG triplets did not have an important function in the nervous system. Research on the triplets in protostomes is still in relatively early stages, but it is clear that they occur only rarely (for example, bees have a single CAG). In most cases, these animal phyla do not carry any CAG in their Huntington's gene.

In the late 2000s our lab analyzed the DNA sequences in the Huntington's gene of other deuterostomes—the most surprising of which was the sequence from the amphioxus, or lancelet, of the Cephalochordata family (which we worked out with Mario Pestarinos's group at the University of Genoa in Italy). The biology of the lancelet, a small, fishlike creature, marks a pivotal development in the evolution of the nervous system—the acquisition of a polarized neural structure extending from front to back in the animal. The front end of this nerve cord in the amphioxus is slightly differentiated to form a sac, or vesicle, which appears to be an early precursor of a primitive brain.

The sequence showed that, as for sea urchins, two CAG triplets occur together. In this case, however, the sequence of genetic letters around the triplet pair was similar to that in vertebrates, including humans, and the protein encoded by the gene was largely confined to neural tissue, allowing us to speculate that this difference might have helped form the primitive brain, with its front-to-back structure.

When researchers then inspected the genomes of vertebrates, they found that CAG triplets begin to lengthen

LAB NOTES

Evolutionary Bequest

An experiment fast-forwards through millions of years of evolution

We have learned from recent experiments that CAG repeats in the Huntington's gene appear to influence the way the nervous system evolved in vertebrates and that more triplets enable a more elaborate early life development process to occur.

In our studies, we looked at the gene's effect on structures called neural rosettes, which arise when cells from the embryo are cultured in a laboratory dish. We re-created the process by working with stem cells taken from early mouse embryos. These embryonic stem cells have the ability to differentiate into other cell types. If the stem cells are treated with molecules known to guide the development of the nervous system, they become what are called neuroepithelial cells arranged around a central cavity in a pattern that resembles a flower—neural rosettes. These rosettes mimic development of the neural tube in the embryo, a structure from which the central nervous system is formed.

First, we showed that the Huntington's gene is important to the rosettes. We found that it allows the cells in the rosettes to adhere to one another. Stem cells deprived of a healthy Huntington's gene did not form the flowerlike structures. In fact, in the absence of the healthy gene, an enzyme cuts the adhesion protein on the cell membrane, preventing the cells from attaching. If the Huntington's gene was restored, rosettes started to form.

Next we asked what would happen if we removed the original gene from a mouse stem cell and replaced it with a gene from the amoeba (no CAGs), amphioxus (two), fish (four) and humans (15), among others? Differences in rosette development suggest whether progressively higher numbers of CAG repeats may render the Huntington's gene more able to help in the formation of the nervous system in these species.

The genes of less complex species, such as the amoeba, did not produce rosettes. The first recognizable structure, albeit incomplete, occurred after inserting the amphioxus gene. In general, genes with more CAGs resulted in better formed and larger rosettes with a large central cavity. The Huntington's gene from fish induced the formation of beautiful rosettes—bigger structures composed of many more cells than those induced by the amphioxus gene. The human gene—the one with the longest number of repeats—yielded the best results, with the largest and best-structured rosettes.

Together the results offer a synopsis of what may have occurred over millions of years of evolution.

—*C.Z.* and *E.C.*

appreciably in organisms with more sophisticated nervous systems until they reach their maximum extension in humans. This can be inferred by looking at species progressively more distant from humans such as cattle (15 CAGs), pigs (18), dogs (10), mice (seven) and opossums (six). Many organisms, including primates, have CAG segments that differ in length among individuals of the same species.

Vertebrates mark a new chapter in neural evolution. Their brain develops from a hollow structure called the neural tube that forms in the embryo and later develops into a brain. In 1997 the group led by Marcy MacDonald of Massachusetts General found that the Huntington's gene is involved in neural tube formation, and in 2012 our team confirmed and extended this finding by showing that it contributes to the development of a neural tube–like structure in a culture dish.

HUMAN TRIPLETS

IN THE MEANTIME, other lines of research began to sketch out yet another role for CAG repeats: improving the mind. These discoveries grew in part out of efforts beginning in the 1970s to look for the gene. Finally, in 1993 geneticist Nancy Wexler and 57 other researchers, all in the Huntington's Disease Collaborative Research Group, isolated and sequenced the human gene, which sits on chromosome 4, thereby paving the way for the discovery that the number of CAG triplets is 36 or more in people with Huntington's.

A year later David C. Rubinsztein, a geneticist now at the University of Cambridge, published a paper suggesting that the CAG section in the Huntington's gene in healthy individuals has a tendency to expand as it is passed on to one's offspring. Also in 1994 Max Perutz, a Cambridge Nobelist, found that glutamine—the amino acid, or protein-building block, encoded by the CAG genetic letters—promotes binding to other proteins. These results, however, were followed by a long lull in research into nonpathological functions of CAG repeats. At the time, CAGs and other duplicated sequences were viewed as genetic "junk," with potentially no purpose.

In 2008 John W. Fondon III, now at the University of Texas at Arlington, and David King of Southern Illinois University Carbondale infused new interest in the question by speculating both that triplet nucleotides might be involved in the development and evolution of the nervous system and that an expansion of the CAG triplet in brain cells may enhance cognition and the capacity for sexual and other forms of social interaction.

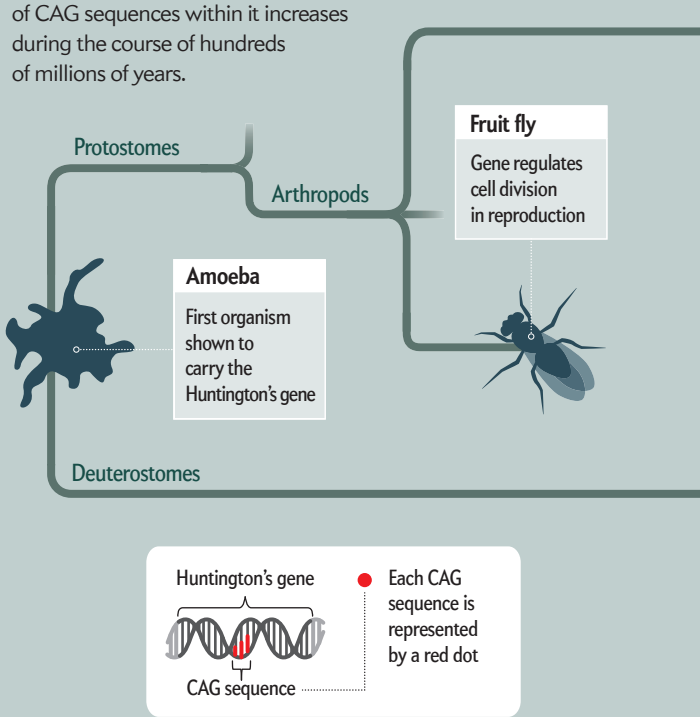
Since then, experimental evidence has mounted in support of these conjectures. According to a study conducted by Michael Hayden's team at the University of British Columbia in Vancouver, one individual in 17 carries an "intermediate allele"—a healthy Huntington's gene with CAGs totaling between 27 and 35 repeats, a high but not pathological number. Healthy people with a high number of CAG triplets tend to have more gray matter (neurons) in the globus pallidus, a brain area that governs the planning and control of movement and is involved in higher-level cognitive processes. In petri dish studies of brain cells, our lab has also shown that more triplets lead to more sophisticated nervous system–like structures [*see box on preceding page*].

Even carriers of the gene who are destined to become ill demonstrate high levels of cognitive functioning. In 2012 Carsten Saft and Christian Beste, both then at Ruhr University Bochum in Germany, reported that those with gene variants that lead to the disease who have not yet developed symptoms achieve better scores in visual and other perceptual tests than people with normal variants.

A TREE OF LIFE

Biography of a Gene

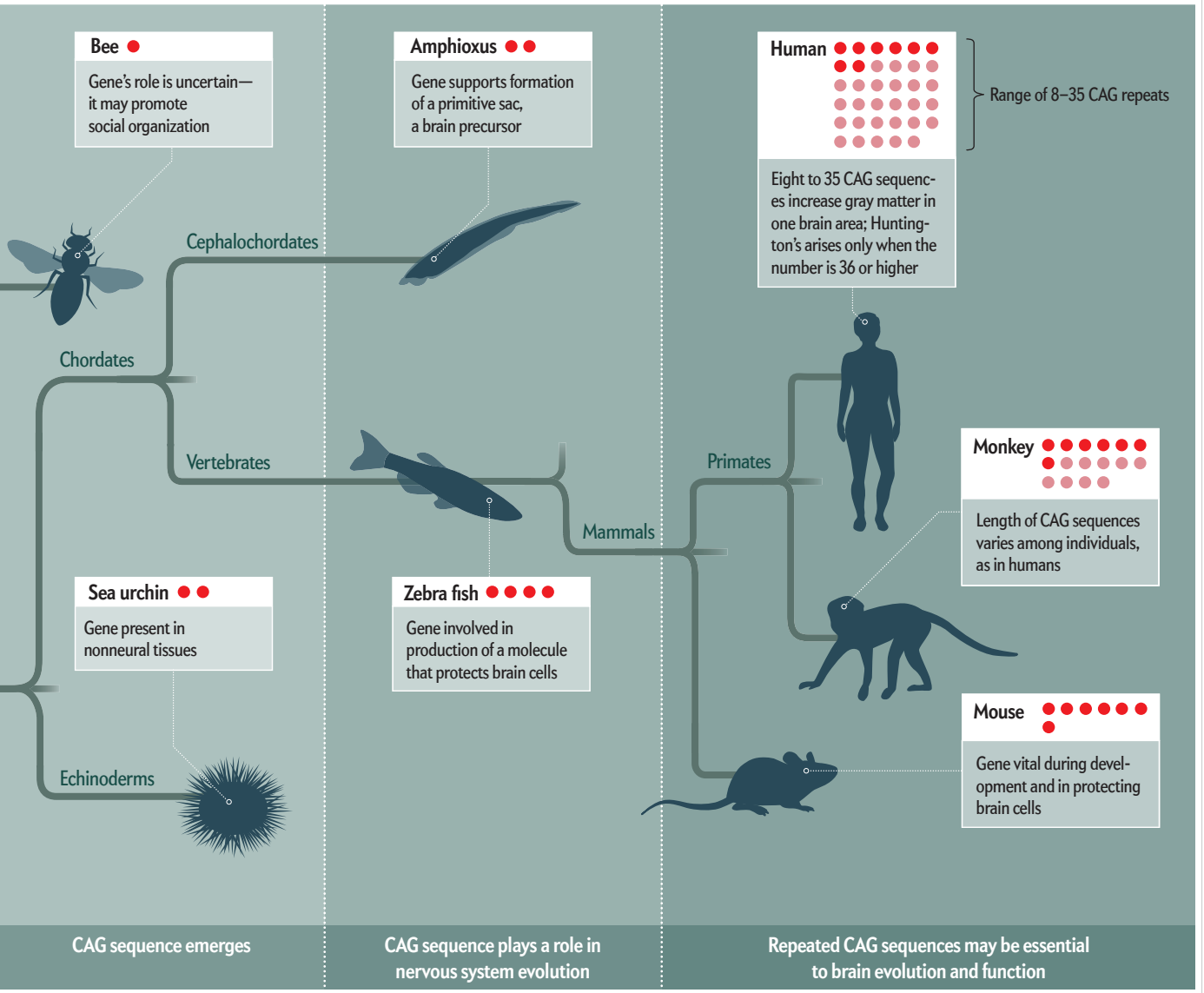
What do we owe to the lowly amoeba? One hand-me-down bequeathed over the eons is the Huntington's gene—the same one that, in its aberrant form, is responsible for Huntington's disease in humans. The unmutated gene appears to contribute to development in early life and to building complex nervous systems. Its story, traced on a tree of life, documents an ever expanding number of biological roles for the gene as the number of CAG sequences within it increases during the course of hundreds of millions of years.



Huntington's gene is present without CAG sequences

A BRAIN HELPER

NEW RESEARCH ON the Huntington's gene has also delved into which specific tasks the gene carries out in the brain. In our research using brain cells in a lab dish, we found that the healthy form of the gene makes neurons hardier and more resistant to stress. Conversely, other researchers found that turning the gene off in the brain of mice causes cells to die and neurological symptoms to appear similar to those seen in mice carrying a harmful version of the Huntington's gene. We have also demonstrated that the gene stimulates the production of brain-derived neurotrophic factor, a protein that promotes the formation of brain circuits and the transmission of nerve signals.



Perhaps most important, the Huntington's gene is in its most active state during early embryonic development. Quite simply, without it, we would not have been born. The gene goes to work during gastrulation, the stage of embryonic development from which the main body tissues develop. Later on, the gene regulates the formation of new neurons and helps to connect them.

Despite the progress made, the paradox of Huntington's persists. Acquiring a CAG tract that continuously extends itself is perhaps the major evolutionary achievement of the Huntington's gene, but the tendency to expand also poses a terrible risk of a devastating disease. Puzzles surrounding the gene's repeating genetic segments will occupy neuroscientists for years to come. We still need a better understanding of why the CAG triplets in the gene vary so much in length. What changes occur in the brain when the number of CAG triplets nears the threshold that will result in a diagnosis of Huntington's? Why does the gene suddenly become harmful at 36 repetitions? Understanding that the Huntington's gene is both a boon and a bane may help allay some of the stigma of the disease, letting it be viewed not as a genetic defect but as an offshoot of a biological process that ultimately made us the human beings we are. SA

MORE TO EXPLORE

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FROM OUR ARCHIVES

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Graphic by Amanda Montañez