



OF MICE AND MEN

Researchers are adding human DNA to mice to pinpoint sequences that helped define our species *By Elizabeth Pennisi*

In 2006, computational whizzes at the University of California, Santa Cruz (UCSC), announced that they had glimpsed the DNA that made us human. They had compared all of the vertebrate genomes sequenced to date and come up with a list of about 50 DNA regions that were identical in many animal species but had changed in humans. Those sequence changes must have been important in the evolution of humans, the team concluded, contributing to our big brains, bipedalism, broad diet, and other traits that have made our species so successful.

By 2008, almost two dozen bioinformatics studies had added hundreds of other uniquely human genetic sequences to the list, and the pursuit of such DNA continues to this day. Yet almost a decade later, not much progress has been made in demonstrating that these DNA sequences—genes, regulatory sequences, and other genome elements—actually shaped human origins. “It’s pretty embarrassing how little we know

about the specific changes in our genome” related to our evolution, says Greg Wray, an evolutionary biologist at Duke University in Durham, North Carolina. “You could imagine [their roles], but they were just sort of ‘just so’ stories.”

Now, with the help of genetic engineering and the humble laboratory mouse, researchers are starting to pinpoint just how some of these sequences might have helped give rise to our uniquely human features. Several groups have gotten clues by inserting pieces of human DNA into mouse embryos, declaring evidence for an evolutionary role if a piece of DNA simply functions in the brain, a limb, or some other structure where humans differ from chimps or other animals. But those studies fail to go the distance. Those studies, however, did not breed mice to have the new DNA incorporated into their genomes, a step that makes it possible to assess how the human sequence affects an organism’s anatomy and function.

By making that effort, Wray and Duke developmental neurobiologist Debra Silver

The blue in the brain of this mouse embryo reflects the activity of a reporter gene driven by the HARE5 enhancer, implicated in human brain evolution.

have recently watched how the human version of a regulatory element called HARE5 altered mice, boosting their brain size by 12%. HARE5 “could easily be a huge component in how the human brain expanded,” says Mary Ann Raghanti, a biological anthropologist at Kent State University in Ohio.

TO WRAY, “the most interesting question is the origin of our species.” The lure of that question drove him and other researchers to their computers a decade ago when sequencing genomes came into its own. First, they compared the human and mouse genomes, and, soon afterward, looked for meaningful differences between humans and other primates, especially our closest relative, the chimp. In 2005, Evan Eichler, a geneticist at the University of Washington, Seattle, and colleagues compared human and chimp genomes to find DNA duplicated in us but not

in our closest relative. They determined that 33% of the duplications in humans were not present in chimps. Four years later, Eichler's team went further, pinning down about 1000 instances in which the human genome has extra copies of genes and regulatory regions called enhancers.

A gene called *SRGAP2*, found as a pair in chimps but in six copies in humans, particularly intrigued Eichler. The initial duplication occurred about 3.4 million years ago, judging from the number of differences between it and the original gene, and a second duplication a little more than a million years later created a shorter gene with a new function. When inserted into the developing brains of mouse embryos, this truncated gene causes cells in the rodent organ to migrate farther and sprout more spines,

they measured how active these genes are in fetal human brain stem cells, the champion was a truncated copy of a gene called *ARHGAP11A*, which was also on Eichler's list. Huttner and his colleagues inserted the copy into the brains of developing mouse embryos and the number of cortex cells nearly doubled. The rodent brains sometimes also developed folds normally seen only in human brains, the group reported online 19 February in *Science*. The truncated copy, they note, is found in the genomes of Neandertals and another ancient human group, the Denisovans, but not in chimps, bolstering suspicions that it played a key role in human evolution.

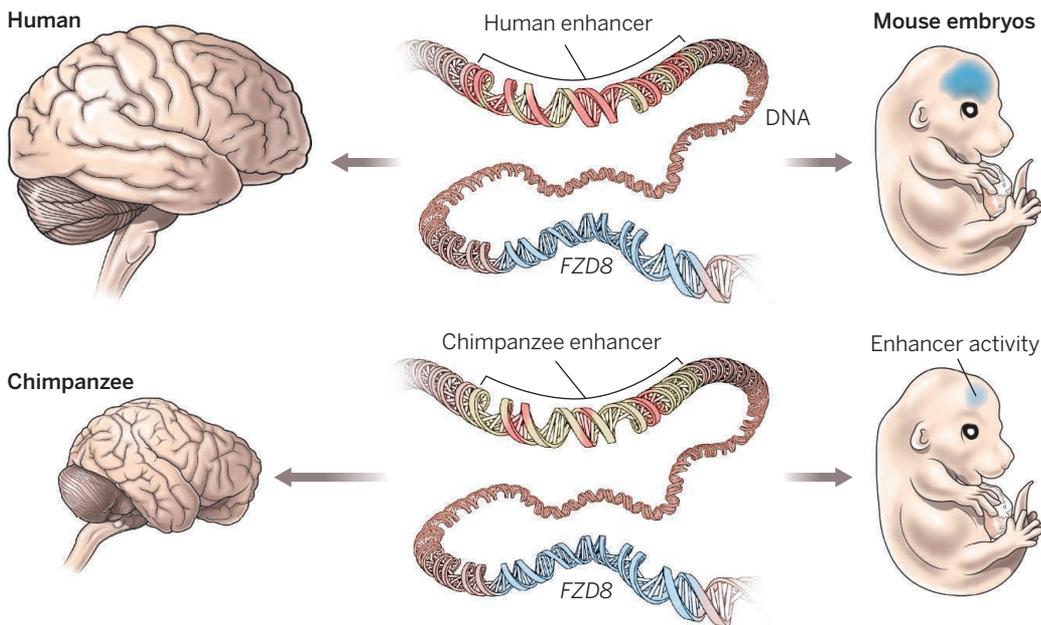
Humans may also have gained brain by losing some sequences. David Kingsley, an evolutionary geneticist at Stanford Uni-

most intriguing is a stretch of DNA identified 9 years ago by David Haussler at UCSC and Katherine Pollard, now a biostatistician at the Gladstone Institutes in San Francisco, California. They called it HARI, for human accelerated region 1, because the human sequence has diverged dramatically, whereas that DNA has remained the same in other animals examined. They determined that HARI encodes a standalone RNA, rather than a protein, and that it is expressed in the developing human brain as nerve cells are forming connections and organizing into layers in the cortex. To many evolutionary biologists, this was exciting, if circumstantial, evidence that the sequence had a role in human brain evolution.

Whereas many of the DNA changes in the spotlight appear to have shaped our brain,

Building a bigger brain

Human brains differ from those of chimps in part because variations in a piece of regulatory DNA called an enhancer cause the human *FZD8* gene to be more active over a larger region of the developing brain, as shown when each species' enhancer is engineered to turn on the mouse version of the gene.



which can increase the number of connections they can make, Franck Polleux, a neuroscientist at Columbia University, and his colleagues reported in 2012 in *Cell*.

Recently, suggestive evidence has emerged that another human-specific duplicated gene also helped increase our brain power. Wieland Huttner, a developmental neurobiologist at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany, and his colleagues identified 56 genes that humans have in multiple copies but mice lack completely. When

iversity in Palo Alto, California, and his colleagues found 500 stretches of regulatory DNA that chimps have but humans do not. One sits near a tumor suppressor gene that keeps cell growth in check. Kingsley is now testing the effect of the deletion in mice, but he suspects that it disables the tumor suppressor and removes the brakes on cell division, allowing extra brain growth in humans.

Along with duplications and deletions, investigators are studying sequences that humans share with other animals—but that show signs of dramatic change. One of the

some may have influenced other human structures. One of the regulatory DNA deletions Kingsley identified may have caused the human penis to lose the spines seen on the penises of mice, chimps, and many other mammals. And another of Pollard's sequences, a DNA segment that is second only to HARI in the number of changes between the human and animal versions, may play a role in the development of the human forelimb, according to a team led by Shyam Prabhakar, now a geneticist at the Genome Institute of Singapore, and James Noonan, now at Yale University. Like Pollard, they and colleagues had pinpointed this sequence as a possible driver of human evolution. When they inserted this regulatory sequence, called HACNS1, into mouse embryos in 2008, they discovered the human version, but not the chimp one, was active in developing rodent forelimbs.

Seven years later, however, Prabhakar and Noonan still don't know what gene or genes

HACNS1 controls, having not yet fully incorporated this DNA into rodents by creating transgenic mice. "Introducing large segments of human sequence into the mouse genome ... is slow, not easily scalable, and expensive," Noonan says. That's been "the limitation for the field overall."

THAT'S THE STEP WRAY finally took. Rather than looking for glimmers of function by inserting a sequence into a mouse embryo, he and Duke graduate student Lomax Boyd set out in 2010 to try to create

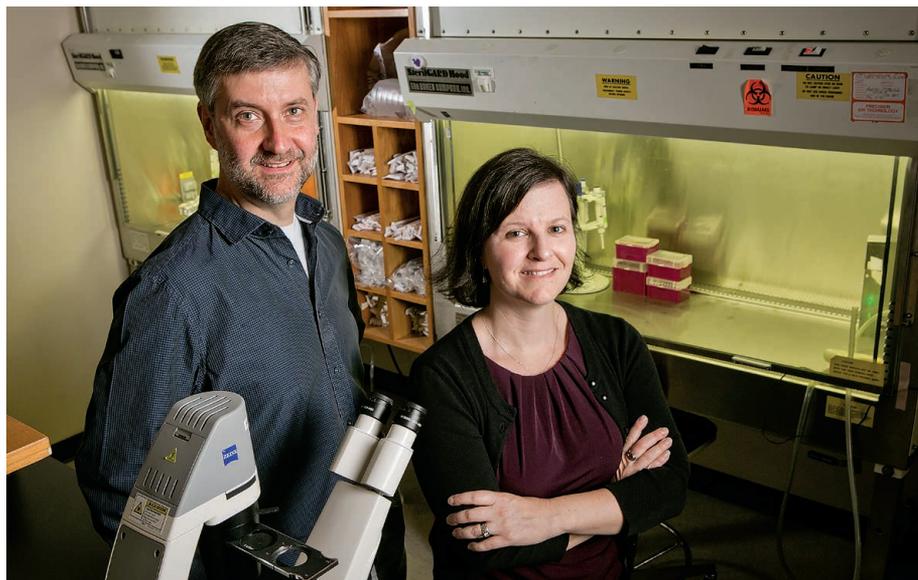
a mouse strain with the human DNA permanently integrated into the rodent's genome. Although new gene-editing technologies promise to streamline such an experiment today, at the time they knew that they faced years of hard work. Because his expertise was in bioinformatics rather than in creating transgenic mice, Wray approached Silver, a new Duke faculty member and a transgenic mouse and brain development expert, about working on the project.

To increase the group's odds of success, Boyd only considered DNA sequences that multiple lists had pinpointed as likely to have been important for human evolution. He also narrowed his focus to sequences that appeared to act as enhancers, because these gene regulators seem to play an outsized role in evolution. For each candidate enhancer, he combed the scientific literature for nearby genes that might be under its control and that either had roles in brain growth or encoded proteins that regulate the activity of other brain-related genes. After about a year, the Duke team had hand-picked nine candidates.

To learn more about their function, Boyd and Silver hooked each candidate enhancer to a reporter gene that would produce a blue color whenever the enhancer was active, then injected the complex into mouse embryos—the type of experiment other labs had done with their sequences of interest. “We use the mouse embryo sort of like a test tube,” Silver says. One enhancer, HARE5, produced a bright, consistent pattern in just the brain of the mouse embryos. In the few mammals studied, HARE5 sits near a gene called *FZD8*, which is part of a well-known pathway that controls mammalian brain growth.

Then came the “money shot,” as Wray calls it—seeing whether the human and chimp versions of HARE5 affected the embryonic mouse brain differently by creating transgenic mouse strains that had one or the other. To remain unbiased, Boyd was blinded to the type of embryo he was analyzing, and he hung pictures of the embryos up in the lab so colleagues could opine on what they saw.

After comparing hundreds of modified embryos at various points during development, Boyd and Silver became convinced that the two HARE5s differed in their timing and level of activity. To confirm, Boyd linked the human and chimp enhancers to genes that produced differently colored fluorescent tags. The team then created a transgenic mouse with the chimp enhancer pairing and a strain with the human DNA, then bred the resulting adults so the offspring carried both types of HARE5s, allowing a direct comparison. The human version turned on



At Duke University, Debra Silver and Gregory Wray used mice to demonstrate how one piece of DNA likely contributed to human evolution.

much earlier and over a broader expanse of embryonic brain than the chimp enhancer.

Finally, the group engineered several strains of mice to carry the human enhancer attached to the mouse version of the *FZD8* gene, the enhancer's suspected target. It was these mice whose brains ended up about 12% larger than those of normal mice or of strains with the chimp enhancer. By studying thin slices of the mouse fetal brains, Silver's team identified the reason. They found that the stem cells that give rise

strong support for the idea. “They have found a smoking gun in the human genome that connects a regulatory element with a proposed pathway for increasing brain size,” says Todd Preuss, a neuroanatomist at the Yerkes National Primate Research Center in Atlanta. Eichler adds: “The molecular dissection is the finest I’ve seen.”

Not everyone is satisfied that the Duke team's work is done. In their experiments, the researchers left the mouse HARE5 intact and added the other versions of the enhancer, but Kingsley suggests a better experiment would have been to completely replace the mouse sequence with the human or chimp version and monitor the effects on *FZD8* and the rodent brain. Boyd tried to do that swap but failed—possibly because the mouse HARE5 is located on a part of the chromosome that was difficult to manipulate using gene-targeting approaches available at the time.

Kingsley would also like to have seen cognitive testing of the big-brained mice. “It would be nice to know if you could make not only a bigger brain, but an animal that's also smarter.” Eichler adds that Silver and Wray could also look for people with brain-size abnormalities to see if they have mutations in HARE5. “That would really be proof of what they are trying to show,” agrees Nenad Sestan, a Yale neurobiologist.

Even with such proof, however, Wray and Silver's mice will never reveal the whole story of how our brains diverged from those of chimps and other animals. “The evolution of the human brain did not occur by a single gene or even a few genes,” Eichler notes. “Rather it was a concert of evolutionary changes.” ■

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to neurons divide 23% faster and generate more neuron progeny when they are equipped with the human HARE5.

The finding, announced 16 March in *Current Biology*, fits with a leading theory about how the human brain evolved. In 1995, Yale University's Pasko Rakic had proposed that the bigger human brain could have resulted from a simple alteration of the cell cycle in progenitor cells—if these precursors divided more often before transitioning to nerve cells, the brain would be much bigger. Rakic and others consider the Duke evidence