Mining the Molecules That Made Our Mind

By comparing the human genome with those of other species, researchers are finding many genes potentially related to brain evolution—but no one is sure which ones helped shape the uniquely human brain.

IN THE 17 AUGUST TORONTO SUN, THE headline trumpeted “A single gene led to humans.” The Independent in the United Kingdom declared “Revealed: the gene that gave us bigger brains.” And in Minnesota, newspaper readers were greeted with “Scientists ID genes that make humans smarter than chimps.” All three stories announced the discovery of a brain-related genetic difference between chimps and humans, and in all three, the newspapers got carried away.

True, the gene featured, called HAR1F, is active in the right place at the right time during brain development to have spurred the expansion of the cerebral cortex, the center for higher cognition in people. HAR1F, which stands for “human accelerated region 1 forward” gene, also shows signs of rapid change since humans and chimps went their separate ways, suggesting that this gene conferred a survival advantage to our ancestors (ScienceNOW, 16 August, scienceconw. sciencemag.org/cgi/content/full/2006/816/2).

Yet HAR1F is only one of about 10 genes to emerge in the past 4 years as potentially key to the evolution of the uniquely skilled human brain. And these discoveries are but the beginning chapters of an epic evolutionary story that we are just starting to read. With the genomes of dozens of species in hand, including human, chimp, and rhesus macaque, as well as powerful bioinformatics methods for comparing and analyzing all this DNA, research into the molecular basis of human evolution has exploded. Scientists using multiple comparative genomic strategies have uncovered hundreds of genes that provide tantalizing clues about hominid evolution. And some of the most provocative finds pertain to brain evolution.

A few researchers, for example, are delving deep into the tree of life, uncovering genetic evidence about when a central nervous system first began to fire from analyses of the genes of simple organisms such as jellyfish. But the scientists grabbing the headlines are the ones finding brain genes that have changed rapidly, duplicated, merged, or boosted their expression since our lineage split from that of chimps. Such genes are prime candidates for crafting the modern human brain.

Many scientists find the potential to understand ourselves irresistible. A rush of comparative genomics results about human evolution are being presented at meetings, and publications are starting to stream out. “Everyone is jumping on the bandwagon,” says Bruce Lahn, a human geneticist at the University of Chicago, Illinois. Progress at identifying the DNA tweaks that distinguish people from other species should be swift. “Within a year or two, we will have a comprehensive list of genome changes to start looking at,” says Christopher A. Walsh, a neuroscientist at Harvard Medical School in Boston.

Emphasize “start.” Despite what the headlines imply, genomic data so far offer provocative clues rather than direct answers to what caused humans to branch out from the primate tree to become walking, talking, creative beings. Tying genomic events such as a gene duplication to human evolution is a challenge, because often researchers know little about what their candidate genes do. To make a concrete connection between a genetic change and the evolution of the human brain “is a much slower process,” says Walsh.

Positive results
Philosophers and scientists alike have long sought to understand what makes humans unique in the animal kingdom. Among other differences, our brain is three times the size of a chimp’s, with a multilayered cortex capable of doing calculus or writing plays. Much of the explanation for our braininess is encoded in the genome, but with 20,000 or so genes to choose from, geneticists have only just begun to come up with effective search strategies to highlight the crucial ones.

One method is to seek genes that natural selection has favored in humans but not in our close cousins, the chimps. Genes that have experienced such positive selection have evolved more quickly than the background rate of evolution. Researchers can spot these speedy evolvers by comparing genes in humans and...
other species. For example, a gene called FOXP2 is mutated in a family with a severe language disorder; 6 years ago, a team led by Svante Pääbo at the Max Planck Institute (MPI) for Evolutionary Anthropology in Leipzig, Germany, found that the human protein encoded by the gene differed from the chimp’s version in two of its 715 amino acids. Given the amount of time since chimp and human ancestors diverged, no such changes were expected. The protein’s alteration may have helped humans develop the fine motor control needed to mouth words, Pääbo suggested (ScienceNOW, 14 August 2002, sciencenow.sciencemag.org/cgi/content/full/2002/814/2).

Pääbo looked for evidence of positive selection on one gene, but Lahn and his colleagues have taken a broader sweep across the genome. They examined 214 genes involved in brain disorders or active only in the brain, comparing humans and macaques and, in a separate analysis, mice and rats. For each pair, the researchers counted both the changes in a gene’s bases that made no difference to the encoded protein—considered the background rate of evolution—and the changes that altered an amino acid. The higher the proportion of protein-altering changes, the faster the gene had evolved.

Overall, Lahn’s team found that evolution in the primate genes was treading along about 37% faster than in the rodent genes. Among just the two primates, human brain genes, particularly those involved in development, were the sprinters, outpacing the number of changes in the equivalent genes in the macaque. Two genes showed particularly strong evidence of selection: ASPM and microcephalin, each of which underlie microcephaly, a genetic disorder that results in a diminutive brain. Lahn and his colleagues reported at the end of 2004.

The “single gene” heralded last month by the Toronto Sun emerged from an even broader search for positive selection—one that covered entire genomes. David Haussler and Katherine Pollard, both bioinformaticists at the University of California (UC), Santa Cruz, and their colleagues developed a sophisticated computer program that matched up the sequenced genomes of multiple species, including chimp, human, rodent, dog, and chicken. The scan, reported online 16 August in Nature, picked out 49 regions in which most genomes shared the same sequence but the human DNA was considerably different, indicating it had changed quite a bit since roughly 6 million years ago when our ancestors split off from other primates.

HAR1F, for example, is part of a DNA sequence that, compared with the other species, has experienced 18 base changes over its 118-base stretch; less than one such base change would be expected over those 6 million years based on the accepted mutation rate for human DNA.

HAR1F codes for an RNA that is never translated into a protein. UC Santa Cruz cell biologist Sofie Salama, working with Pierre Vanderhaegen, a neuroscientist at the University of Brussels in Belgium, has found that the gene is very active in the developing brains of 2-month- to 5-month-old human embryos. The RNA exists in cells that organize the human cerebral cortex into layers, and because RNA can play a role in gene regulation, Pollard and her colleagues suspect that HAR1F’s RNA helps control the production of proteins involved in cortex development and that a change in its regulatory abilities prompted a larger, more complex cortex.

Key expression

That HAR1F’s possible role in differentiating the human brain from the chimp’s involves regulating other genes should not be surprising. There are relatively few differences in the proteins encoded by the two species’ genes—FOXP2 being one exception—and researchers have long suspected that changes in where, when, and how much a gene is expressed may instead be the real key to our uniqueness.

To understand the evolutionary importance of changes in gene activity, some genomicsists have simply looked for genes that are more active in one species than in another. Others have sought out extra copies of genes and other DNA that might also affect gene expression.

Pääbo pioneered the former approach in 2002 when he, Wolfgang Enard, also of the MPI for Evolutionary Anthropology, and their colleagues compared the overall amount of messenger RNA (mRNA) produced in various human, chimp, orangutan, and macaque tissues, including gray matter. “This was the first attempt to use high-throughput technology to address this very important issue,” says Xun Gu, a molecular evolutionist at Iowa State University in Ames.

Pääbo’s crew used a microarray to detect the mRNA concentration of 12,000 genes. Overall, Pääbo and his colleagues found that genes in the brain tend to have undergone more changes in expression—increases or decreases relative to the chimp—compared with genes in other organs since the two lineages diverged. But they saw little such gene-expression variation between brain and other organs when comparing the chimp with other primates. The result suggested that altered gene activity played a role in distinguishing the human brain from those of its cousins, Pääbo and his colleagues reported (Science, 12 April 2002, pp. 233, 340).

Gu’s team later took a closer look at Pääbo’s data and found a trend in the human versus chimp gene-expression changes in brain. The human brain genes typically had greater activity than their chimp counterparts. “We demonstrated that evolutionary changes may be mainly caused by a set of genes with

“Making room. Skulls (top to bottom) of the human, chimp, orangutan, and macaque housed brains weighing 1350, 400, 400, and 100 grams, respectively.”

Svante Pääbo, Max Planck Institute for Evolutionary Anthropology

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Svante Pääbo, Max Planck Institute for Evolutionary Anthropology
Building the Body from Genes

increased rather than decreased expression,” says Gu.

Mario Cáceres of the Salk Institute for Biological Studies in San Diego, California, and his colleagues have reached a similar conclusion. They’ve used mRNA assays to compare gene expression in the cerebral cortex of humans, chimps, and macaques, finding 91 genes that had changed their activity level since chimps and humans went their separate ways evolutionarily. About 80 of those became more active in the human brain, they noted in 2003.

Double the dose

None of these original expression studies tried to pin down the cause of the increased gene activity. But studies by James Sikela, a genome scientist at the University of Colorado Health Sciences Center in Aurora, Evan Eichler of the University of Washington, Seattle, and others offer one potential explanation: The human genome has plenty of extra copies of genes and other DNA sequences.

In 2004, Sikela, Jonathan Pollack of Stanford University in Palo Alto, California, and their colleagues did a pioneering full-genome scan of five primates, including humans, and found 1005 genes that had increased or decreased in number in one of the species after it split off from the ancestral primate tree. Of those, 134 had undergone duplication in the human lineage.

The next year, Eichler and his colleagues completed genomewide catalogs in humans and chimps of so-called segmental duplications, pieces of copied DNA that range in size from a few thousand base pairs to large sections of chromosomes. Eichler’s group found that the genomes have separate duplication histories. Overall, duplicated segments in the chimpanzee outnumber those in human, but in humans, there’s a greater variety. More genes appear multiple times in our DNA than in chimp DNA. By one count, humans have extra copies of 177 full and partial genes. Each of those genes is a candidate for having altered expression patterns, and Eichler has been investigating the function and activity of several duplicated regions.

The availability of multiple copies of a gene provides more than just a way for a gene to produce additional amounts of its protein or RNA. These extra copies are material with which evolution can play, perhaps dedicating the activity of one copy of a gene to a subset of cells, for example. “The likelihood of innovation is much higher,” says Eichler.

In some cases, such innovation could come from parts of a gene that proliferate instead of a whole gene undergoing duplication. Sikela, Magdalena Popesco, a molecular geneticist at the University of Colorado Health Sciences Center, and their colleagues have recently discovered a stretch of DNA, consisting of just two exons from a gene, that has increased from a single copy in mice to 212 copies in humans and may have evolved crucial new functions in the process.

Sikela’s previous work had identified 134 genes that duplicated primarily after human ancestors split off from other primates. Popesco, Sikela, Gerald Wyckoff of the University of Missouri, Kansas City, and their colleagues compared the sequences of these genes in primates, mice, and rats, and one gene in particular stood out: MGC8902. Humans have 49 copies of this gene, whereas chimps have 10 and macaques have four, the group reported in the 1 September issue of Science (p. 1304).

No one knows exactly what the gene does, but a closer look revealed that it primarily consists of six copies of a two-exon segment that encodes a protein domain—a peptide that has a particular fold or twist—called DUF1220. This domain also has no known function, but the DNA for it is sprinkled liberally throughout the human genome, with the two-exon segment showing up in about two dozen different genes. These genes are active throughout the body, but Popesco notes that they are particularly busy in neurons in the cortex, suggesting that the domain is important in the brain. “The work highlights the potential importance of duplications in the emergence of novel genes within the hominoid lineage,” says Eichler.

Another intriguing human gene apparently arose when part of one gene replaced part of another. While comparing the human and chimp genome, glycoprotein biologist Ajit Varki of UC San Diego had noticed that the front end of a gene called SIGLEC-11 was quite different between the two species, whereas the back end was virtually identical. The front end of the human version turned out to come from a gene called SIGLEC-16, which appeared as a duplicated copy of SIGLEC-11 about 15 million years ago. SIGLEC-16 is now dysfunctional in both the human and the chimp, suggesting that it lost function before the two lineages split.

However, at some point in hominin evolution, part of SIGLEC-16 must have replaced the front part of the DNA of SIGLEC-11, creating a “brand-new, ‘human specific’ protein,” says Varki. The gene for this protein now turns on in the human brain, specifically in microglia, cells known to be important to the growth of nerve cells (Science, 9 September 2005, p. 1693). The finding is “tantalizing, but of uncertain significance,” Varki points out.

Finding function

Varki’s measured summary of the SIGLEC-11 story would be apt for many of these headlining grabbing genes. Whether it’s FOXP2, HARP1, or the DUF1220 domain, “there’s a tendency for people to think this [gene] is it”—the explanation for why people are unique, says Pääbo. For his part, Varki warns that the quest to understand human evolution is too brain-centric. “We are also defined by differences in our reproductive, musculoskeletal, and immune systems, as well as by our skin,” he points out.

Moreover, almost every gene linked to human brain evolution comes with questions
about the strategies used to establish the connection. For example, some scientists are not convinced that the methods used to detect positive selection in a gene are reliable or that the results will hold up as more genome sequences are added to these analyses. Genes that appear to change rapidly may still fall within the range of normal variation, for example. Moreover, rapid change in a gene or extra copies "does not mean that these genes are [all] important during the evolution of the human brain," says Gu.

Some scientists also question the relevance of certain findings, such as a protein-coding gene’s mRNA being produced in a brain region. After all, not all mRNAs are translated into proteins. "I wouldn’t put much stock in claims about genes being expressed or not expressed in the brain unless there’s direct experimental evidence" that the genes’ proteins are actually made, says Todd Preuss, a neuroscientist at Emory University in Atlanta, Georgia.

Eichler illustrates the challenges the field faces when he complains about spending the past 5 years trying to figure out the role of a promising family of genes that emerged from his survey of segmental duplications. The family is in one of the most rapidly evolving duplicates. The genes are about 12 million years old and have undergone rapid evolution in humans, chimps, and gorillas. But the gene doesn’t exist in mice, and the human copies and their surrounding DNA are too similar to track individually in disease studies.

Pääbo shares Eichler’s frustration at the field being unable to move more swiftly beyond highlighting candidate brain-evolution genes. “It’s getting a little stale,” he admits, “to say, ‘I have another case of positive selection.’ The challenge is to link the evidence of positive selection to brain function.”

That may be starting to happen in a few select cases. Take the microcephaly genes, ASPM and others, which may provide clues about how the human cortex got so big. Wieland Huttner of the MPI of Molecular Cell Biology and Genetics in Dresden has demonstrated how the amount of ASPM affects brain growth in mouse embryos. He studied mouse embryonic neuroepithelial cells, the stem cells that give rise to neurons. The longer these stem cells remain undifferentiated, the more they divide and the more neurons that ultimately form.

In anticipation of cell division, ASPM concentrates at opposite ends of the cell and helps organize the microtubules that pull duplicated chromosomes apart. When Huttner reduced the cell’s cache of ASPM, cells no longer divided symmetrically. Instead of forming two daughter stem cells, one of those “daughters” specialized as a neuron, short-circuiting the expansion of the cortex, his team reported in the 5 July Proceedings of the National Academy of Sciences.

Researchers have found that two other genes associated with human microcephaly are active during cell division as well. Last year, Jacquelyn Bond of the University of Leeds in the U.K., C. Geoffrey Woods of the University of Cambridge, and their colleagues used mice to show that cyclin-dependent kinase 5 regulatory protein (CDK5RAP2) and centromere-associated protein J (CENPJ) are active in the same cells affected by ASPM, the embryonic neuroepithelial cells of the frontal cortex. Both the CENPJ and CDK5RAP2 proteins colocalize with ASPM during cell division, Bond, Woods, and their colleagues reported. Earlier this year, Lahn’s team showed that the genes for these two proteins have been evolving rapidly in the human lineage, similar to ASPM.

Another somewhat developed scenario for human brain evolution centers on the DNA that regulates a gene called prodynorphin (PDYN). The protein encoded by the gene is a precursor for opiate compounds important in perception, pain, social behavior, and learning and memory. In rats, for example, increased production of prodynorphin in the brain translates into higher pain thresholds. The gene’s activity is under the influence of a 68-base DNA sequence. Greg Wray, an evolutionary biologist at Duke University in Durham, North Carolina, and his colleagues have found that humans have up to four copies of this regulatory stretch, whereas monkeys and other great apes only have a single copy. Moreover, the researchers have found five base changes in this regulatory DNA that have occurred since the split between chimps and humans, a sign of accelerated evolution.

In the lab, nerve cells with the chimps version of the regulatory sequence make less prodynorphin, Wray’s team reported in the December 2005 PLoS Biology. That same regulatory DNA from the rhesus macaque, gorilla, and bonobo also failed to stimulate adequate prodynorphin mRNA production in human nerve cells. It seems that “humans have evolved to make more of this key brain peptide,” Wray concludes. His team is now reconstructing the ancestral sequence of this piece of DNA.

The prodynorphin example is one of the most advanced, but even this evolutionary story is unfinished, because no one knows exactly what effect the extra prodynorphin has in the human brain, says Wray. And most other examples are in even earlier stages. “In virtually all cases, the link of genes or genomic patterns with human brain evolution is only tentative and based on suggestive evidence,” says Lahn. “The situation may not change anytime soon due to the complexity of the questions and because we can’t redo the experiment that evolution did in many millions of years.” Headline writers, pay heed.

—ELIZABETH PENNISI