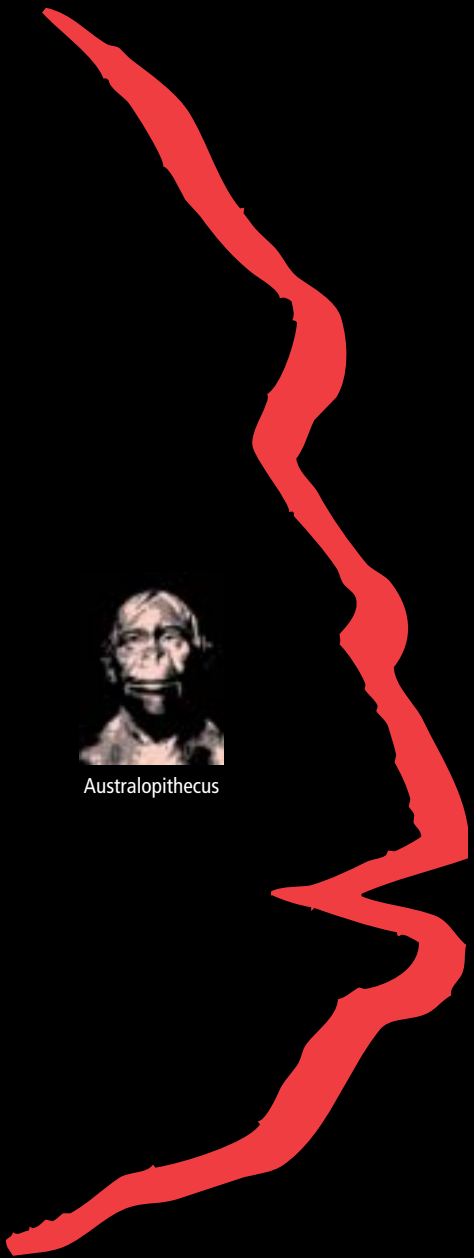


R O O M F O R



Australopithecus



Rhodesian Man



Cro-Magnon Man

T H O U G H T

By Lisa J. Bain

A multidisciplinary team at Penn has discovered a mutation in a muscle gene that may have led, nearly 2.5 million years ago, to the smaller jaw muscles and larger brains of humans. The mutation, they argue, lifted a constraint that had inhibited growth of the brain case.

When Hansell Stedman, M.D., G.M.E. '94, stopped Nancy Minugh-Purvis in the garage one November day and said he had something to show her in the lab that he thought would interest her, she was immediately intrigued. Stedman, an associate professor of surgery and muscle biologist, and Minugh-Purvis, a paleoanthropologist and developmental anatomist in the Department of Cell and Developmental Biology, might seem an unlikely match as research collaborators. But they had found that they had some common interests early in their careers – when Stedman was a postdoctoral fellow studying muscle proteins in the Human Genetics and Anatomy lab of Neal Rubinstein, M.D. '73, Ph.D., associate professor of cell and developmental biology, and Minugh-Purvis was teaching gross anatomy with Rubinstein. Says Minugh-Purvis, “Hansell was always interested in evolution. Years and years ago he was always sending me an article here or there and asking a lot of questions.” So the intersection of his work in muscle biology with hers in paleoanthropology seemed natural, albeit somewhat unusual.

Larry Kaiser, M.D., chair of Penn's Department of Surgery, puts it more succinctly. “A surgeon doing this kind of work is unique. Stedman's a unique guy.”

Minugh-Purvis, it seems, is also somewhat unusual for a person trained as an anthropologist. (She received her Ph.D. degree from Penn in 1988.) In the late 1980s, she was recruited to the Children's Hospital of Philadelphia by Linton Whitaker, M.D., G.M.E. '71, professor of surgery at Penn and chief of the division of plastic surgery. Whitaker wanted someone with an anthropology background who could do growth evaluations on pediatric patients with craniofacial anomalies. But about seven years ago, Minugh-Purvis says, she reached the point of wanting to move beyond the paleontological aspects of her work and to begin looking at the molecular biology of bone and cartilage. Now she finds herself at the vanguard of a new generation of paleoanthropologists who rely on the techniques done in molecular biology laboratories to help them explain and understand what they see in the fossil record.

“I probably should have been a biologist all along,” she says with a laugh. “But the trouble is that human evolution is really fun stuff, and once you get hooked you're

Part of the research team led by Hansell Stedman and Nancy Minugh-Purvis, front, included (from left to right) Ben Kozyak, medical student; Danielle Thesier, graduate student in Biomedical Graduate Studies; and Marilyn Mitchell, senior research specialist.



in trouble. I still love it, but I really do feel that in order for people to get some of the answers to these very long-standing questions in paleoanthropology, they have to go molecular.”

The research findings that Stedman shared with Minugh-Purvis further intrigued her. His project was already multi-disciplinary, drawing expertise and inspiration from the fields of muscle biology, genetics, and evolutionary genetics. With Minugh-Purvis on the team, the project would stretch to include developmental craniofacial biology and gross and comparative anatomy, as well as an in-depth look at the fossil record. As Minugh-Purvis sees it, the Penn environment facilitated their collaboration: “This is *the* place to be. It’s very hard, particularly right now, to dovetail so much breadth and to do interdisciplinary work like this because you have to be such a specialist. I was lucky to work with Hansell because he really has a lot of breadth and is interested in pursuing questions outside of his immediate area. So it was a happy meeting of the minds.”

And speaking of minds, what Stedman and Minugh-Purvis eventually published earlier this year in one of the world’s most prestigious science journals was a study of a mutation that undermines an entire gene of myosin, the major contractile protein that makes up muscle tissue. The newly discovered mutation appears to be the cause for the development of smaller jaw muscles in humans as compared to non-human primates. Did smaller jaw muscles ultimately remove the constraints on the development of the human brain?

The long search for mutant muscle proteins begins

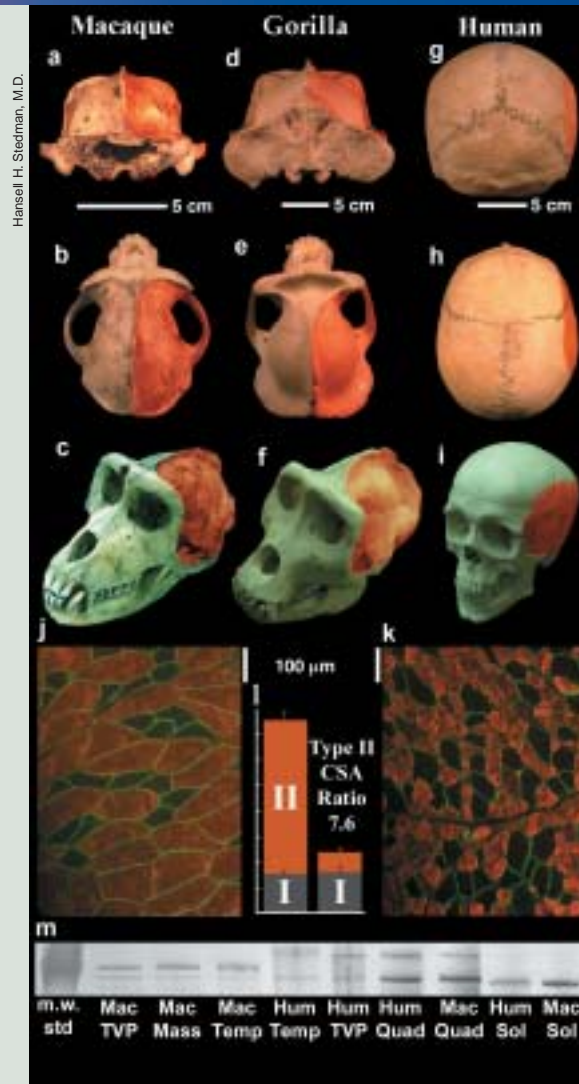
The project began more than 12 years ago when Stedman was a research assistant professor and midway through his residency. He had become interested in muscle biology even earlier, in part as a

consequence of growing up with two brothers who had Duchenne muscular dystrophy, as well as with a mother who fueled his interest in science. He recalls a time during his undergraduate years at M.I.T. when he came home for summer vacation. His younger brother, then in his late teens, was working on a high-school science project in human genetics. Says Stedman, “He had some unbelievably profound things to say, especially for a guy who had been in a wheelchair for five years, about Darwinian selection, survival of the fittest, experiments of nature, and how it all fit together.” His brother asked for advice about a project he was doing involving an animal model of muscular dystrophy. “It was a huge eye-opener for me – first of all, because of the mere fact that he was thinking outside the box.” Stedman started asking his own questions about muscle biology, which led him to John C. Seidel, M.D., a muscle biochemist who was studying myosin. Seidel convinced Stedman of the wisdom of combining a basic research focus on how muscle works with an applied focus on what happens when the muscle does *not* work.

Thus, Stedman found himself in the early 1990s with about one year to accomplish something significant in the research lab before returning to a surgical residency. There was an enormous incentive, he says, to come up with short cuts. “And we hit on a way to make a slam dunk DNA test for all of the myosin genes that create muscle motion in any organism whose DNA we could isolate.”

The central role of myosin

Myosin, according to Stedman, is one of the two most important molecules of life; the other is ATP, which he described as the energy currency for life. Myosin, explains Stedman, powers all locomotion as we know it in all animals of the biosphere. Drawing an analogy with the au-



Hansell H. Stedman, M.D.

tomobile, he calls ATP the gasoline and myosin the piston. Furthermore, not only is the interaction of myosin and ATP absolutely critical for survival, Stedman continues, it is a major source of dietary protein. “When you’re on the Serengeti plain in a fight-or-flight struggle, if you win the predator-prey interaction, myosin is a big part of the payoff.” And since myosin is hugely abundant in muscle, defects in myosin could be responsible for many muscle diseases, although it is now known that mutations in another protein, dystrophin, are responsible for the various muscular dystrophies.

Stedman’s team used what was, in 1990, a novel technique called polymerase chain reaction, or PCR, to find ten different myosin genes in the human genome. The technique allowed them to find pieces of DNA that had sequences similar to previously published myosins, but told them nothing about whether these genes were actually expressed or where they were located in the genome. Stedman’s team put the project on hold while another project studying muscular dystrophy in the mouse occupied most of their attention. Over the next ten years, the muscular dystro-

phy research progressed to the point where a gene therapy trial was planned. But the tragic death of Jesse Gelsinger in a human gene therapy trial at Penn in 1999 brought much of the gene therapy research to a halt. As Stedman puts it, “It was that event and its downstream consequences that in no small way convinced us to look back at some things that had been put on the back burner for a while.” Fortunately for him, his team had been strengthened by the addition of a couple of rookies with time to explore new ideas. Benjamin Kozyak, a medical student, and Anthony Nelson, an undergraduate from Drexel University, worked with Stedman under the joint mentorship of Joseph D. Shrager, M.D. ’60, G.M.E. ’64, chief of cardiothoracic surgery at HUP, and Charles R. Bridges, M.D., Sc.D., G.M.E. ’91, chief of cardiothoracic surgery at Pennsylvania Hospital. Other team members contributed expertise and advice in molecular biology and computer algorithms, but it was Marilyn Mitchell, Stedman’s most senior research specialist, who made it possible for Kozyak and Nelson to do all of the molecular biology, including some long and tedious protein fractionations that ultimately isolated the proteins they were studying.

Among the 10 sequences they had identified in 1990 was one that looked so different from the others that the researchers could not make sense of it. “As recently as two years ago, when we realized we had cloned and recognized yet another myosin, we didn’t know what to call it other than give it a number, so it’s MYH16 because it’s the 16th one discovered,” says Stedman. “We thought maybe it was a candidate gene for yet another muscle disease in humans, and we would get

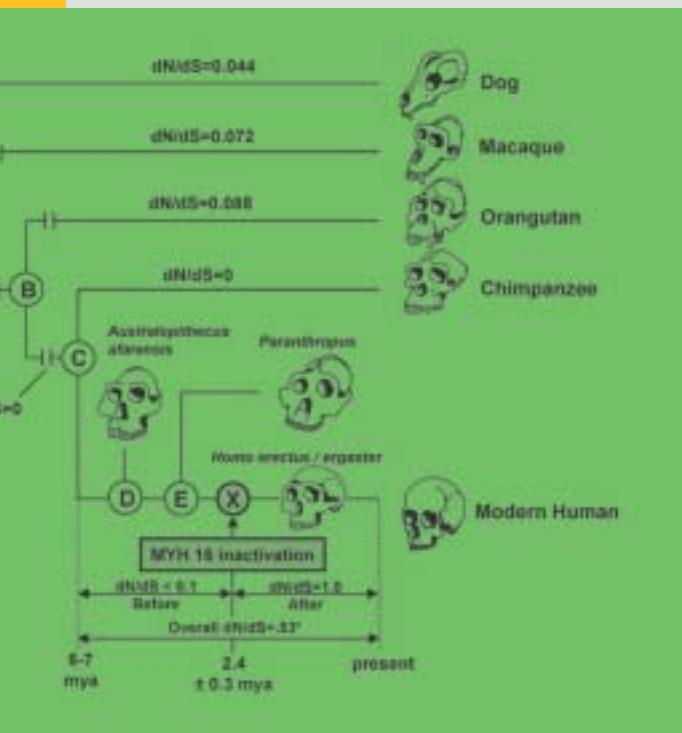
back to it when we figured out which one. It didn’t dawn on us then that it was going to be the most common of all human diseases. We all have it.”

One of the first questions they needed to answer was how this gene related to other known myosin genes. Using draft sequences obtained through the continuing human genome project, they lined up the sequences for various myosin genes and discovered a two-base pair “glitch.” Two links in the DNA chain found in all other known myosin genes were missing in MYH16. What was particularly noteworthy is that the change in the sequence would make the gene non-functional, unable to code for usable protein.

One possible explanation for this glitch was that the anonymous person whose DNA had been used for the human genome project had a rare mutation. But when they looked at other human DNA samples from geographical locations as distant as Africa, Iceland, South America, Western Europe, Japan, and Russia, all were found to have this same mutation. Yet, to the researchers’ amazement, the MYH-16 sequences from all non-human primates showed no deletions; all of these species, that is, have the blueprint for a normal myosin protein. Among the non-human primates, there was some genetic drift as the species diverged further apart on the evolutionary tree, but the drift appears to be in spacer (or “junk”) regions of the DNA, not in the regions that code for myosin protein. That distinction indicates that the gene was under enormous evolutionary constraint. In Stedman’s vivid description, “One trip off that tightrope buys you a real problem.”

The answer is in the bite

Next, the researchers wanted to know where this gene is turned on. To answer this question, they obtained muscle tissue specimens from autopsies of non-human primates that had been involved in other



studies. What they found was that the gene was only turned on in the powerful biting muscles of the jaw. A comparison of the jaw muscles of humans with those of macaque monkeys revealed even more clues about the consequences of this genetic mutation. The jaw muscle of macaque monkeys is composed predominantly of enormous muscle fibers called “fast twitch” muscle, along with some smaller fibers called “slow twitch” muscle fibers. In contrast, human jaw muscle contains similarly sized slow twitch fibers, but much smaller fast twitch fibers than those seen in the macaque. What that means is that the macaque jaw muscle is about eight times larger than the equivalent human jaw muscle; at the same time, other muscles such as those in the leg are similar sized, whether human or monkey.

Stedman was struck by the difference and the implications it could have on human evolution. He was particularly intrigued by the shape of the skulls of non-human primates compared with humans and how the shapes related to the difference in jaw muscles. It was then that he turned to Minugh-Purvis. While anthropology has held a particular fascination for Stedman over the years, Minugh-Purvis brought to the table an intimate hands-on familiarity with the primate fossils. Says Stedman, “It’s been like getting a drink from a fire hose being around her and learning all this stuff.”

Gorillas and other non-human primates have distinct mid-line bony crests and prominent cheekbones. While these serve to anchor their massive jaw muscles, they also may restrict expansion of the skull and thus impose limitations on the growth of the brain. Humans, in contrast, lack the bony midline crests and large cheekbones; their skulls are designed to expand as the brain grows during infancy and childhood. So the questions Stedman laid before Minugh-Purvis: Might the lack of massive jaw muscles resulting



Pooling their expertise allowed Stedman and Minugh-Purvis to reach a compelling hypothesis.

from the MYH16 mutation permit expansion of the brain case and, thus, permit growth of the brain itself? And could this mutation, at least in part, explain the evolutionary leap from ancestral apes to early humans?

Minugh-Purvis found Stedman’s ideas compelling, particularly because he had come up with an idea that could be tested in the laboratory. Paleoanthropologists never get the opportunity to test their hypotheses in the fossil record, she says, but using the techniques of genetics and molecular biology in animal models, one could manipulate the muscle and then study the effect on the bone development. In fact, one such study has already been done in mice; it showed that, indeed, muscle can sculpt bone. When a mutation was introduced in a muscle protein, it dramatically affected bone development.

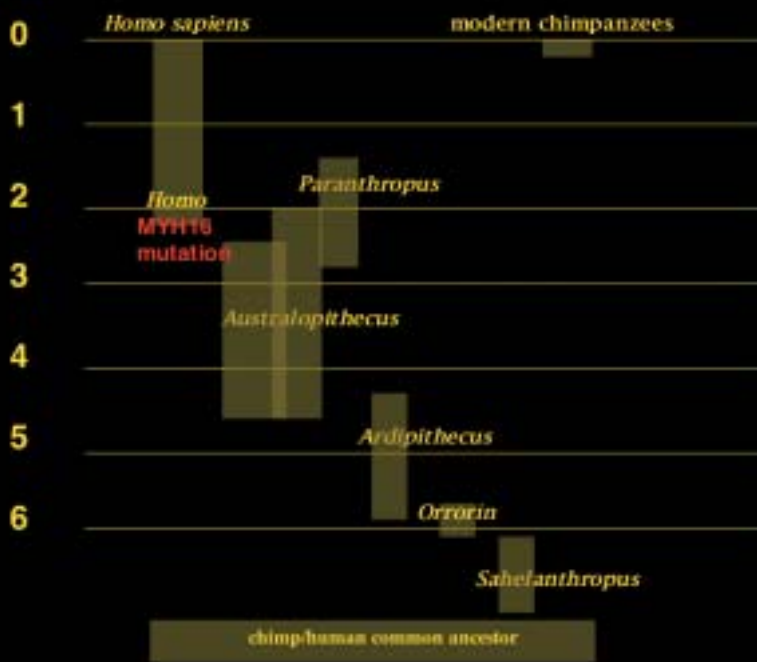
One of the first things Stedman asked Minugh-Purvis to do was to study the fossil record to try to determine when this mutation might have occurred. Meanwhile, he consulted Warren J. Ewens, Ph.D., a professor of biology at the University, and colleagues in Penn’s Center for Bioinformatics to fix a time for the mutation

using the “molecular clock,” a method that analyzes sequence variations across species in a common lineage. As Minugh-Purvis recalls, “I came back to him and said, ‘How about between 2 and 2 1/2 million years ago?’ And he said, ‘Bingo!’ His calculations had gotten him to 2.4. So we were very excited that they coincided.”

Clues in the fossil record

The Rift Valley in East Africa is home to one of the richest sources of hominid fossils dating from about two million years ago. Living essentially contemporaneously were two very different species: *Paranthropus boisei*, known as a robust australopithecine; and *Homo ergaster*, a predecessor to *Homo erectus*. According to Minugh-Purvis, the distinction between them is “absolutely profound.” *Paranthropus* has a crested skull and widely flaring cheekbones to which the enormous biting and chewing muscles could attach. *Homo*, meanwhile, has no midline crest and the cheekbones are compressed against the sides of the face, giving it a much more human appearance. “In sum total,” says Minugh-Purvis, “you would say clearly that the masticatory system, the biting muscles involved

MYA



in chewing, are considerably reduced in the early *Homo erectus* specimen.”

What’s more, the *Homo* specimen has a brain case that is about 70 percent larger than that of *Paranthropus*. In fact, starting about two million years ago, the human brain has essentially tripled in size. “As an evolutionary trend, the tripling in size of the human cranial capacity is almost an unprecedented phenomenon,” says Minugh-Purvis. “Paleoanthropologists have always themselves marveled at the fact that this could have happened in such a short time period.”

Another compelling piece of evidence, as Stedman points out, is that there are no specimens in the fossil record that have both a large skull and a midline crest and flared cheekbones. “It’s either/or. Our feeling is that something had to give.” The massive jaw muscles seen in all predatory species, including non-human primates, would crush the skulls of the animals were it not for the enormous deposition of bone to house the muscle. A mutation that reduced the size of these muscles could, however, reduce the need for the additional bone and allow for the expansion of the skull.

“We’re not suggesting that this muta-

tion alone buys you *homo sapiens*,” says Stedman. “But it’s much more reasonable to conclude that it lifted an evolutionary constraint that facilitated the accumulation of additional mutations that sustained brain growth.” Moreover, in order for the mutation to become fixed, as indeed happened, it would have had to confer a selective advantage to its host.

“The fact that it became fixed suggests that the hominids who had it weren’t dependent on massive chewing muscles already, because the earliest stone tools that are recognizable were 2.6 million years ago,” says Minugh-Purvis. “But you are still confronted with the question of why did it become fixed. And that’s where we are now.”

Putting it all on the table

Stedman and Minugh-Purvis published their study in the March 25, 2004, issue of *Nature*, generating significant press coverage as well as controversy. Pete Currie, a developmental biologist from Sydney, Australia, wrote in the accompanying commentary that Stedman et al. described “what may be the first functional genetic difference between humans and apes” and presented “convincing evidence” of how

this mutation could have been responsible for the acquisition of “human-like” characteristics. In *The Washington Post*, Milford H. Wolpoff, a University of Michigan paleoanthropologist, was quoted as saying, “I love this paper. It’s perfect.” In *Science*, Ajit Varki of the University of California at San Diego asserted that the work by the Penn team “represents a significant advance. It’s the first example of a defined [protein] difference between humans and great apes that results in a functional consequence.”

Other anthropologists and evolutionary geneticists expressed more skepticism, arguing that the explanation is too simple. *Science* also quoted one of the most vehement critics, Ralph Holloway of Columbia University: “To suggest that the brain is constrained by chewing muscles is just rubbish.” Stedman, however, is undaunted by the criticism, acknowledging that “this is going to take at least 10 years to nail down.” He points out that James Watson, co-discoverer of DNA, said that he and his colleagues were out on a limb for five years with DNA before the scientific community was ready to accept their ideas. Says Stedman, “They took a lot of criticism because experimental work hadn’t been done that could nail it down.”

In the meantime, Stedman, Minugh-Purvis, and the rest of their team are continuing to study the differences in muscles and bones of non-human primates compared to humans, as well as how alterations in muscle proteins affect the development of the bones to which they attach. “I’ve gotten thousands of e-mails about this thing,” Stedman reports, “and the general amount of feedback suggests that this has captured peoples’ imaginations. That’s why we jokingly call it the ‘room for thought’ mutation.” ■

Lisa J. Bain’s article on H. Lee Sweeney appeared in the Spring 2004 issue of Penn Medicine.