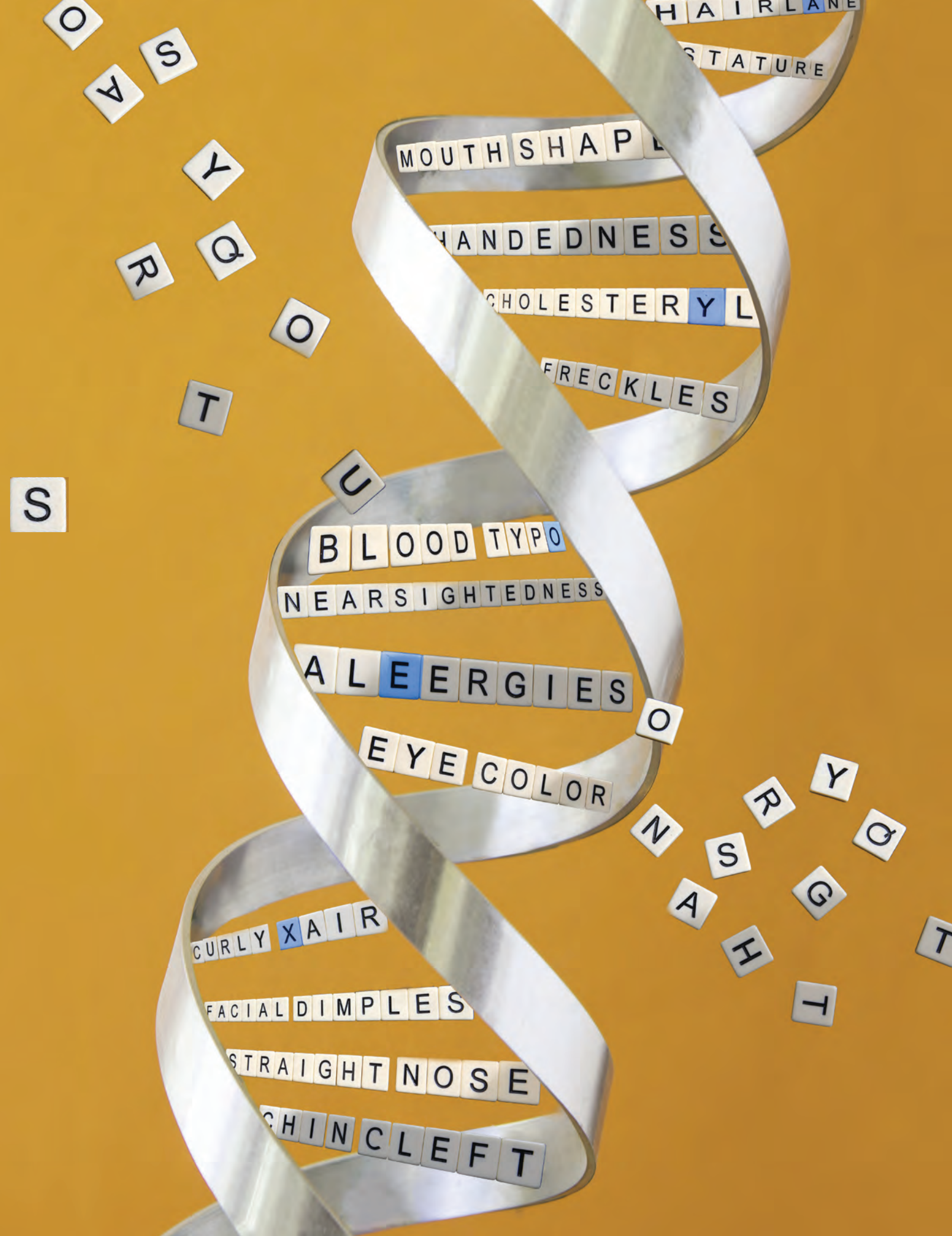
HOW THE QUEST TO UNDERSTAND HUMANITY'S SHIFTING BLUEPRINT MAY BETTER ARM US TO FIGHT CANCER AND PREVENT BIRTH DEFECTS

BY IAN DEMSKY


HAIRLANE
STATURE
MOUTHSHAPE
HANDEDNESS
CHOLESTERYL
FRECKLES
BLOOD TYPO
NEARSIGHTEDNESS
ALLERGIES
EYE COLOR
CURLY XAIR
FACIAL DIMPLES
STRAIGHT NOSE
CHIN CLEFT

A FAMOUS proverb holds that Persian carpets are perfectly imperfect, precisely imprecise. Intentional flaws in the weave symbolize a belief that true perfection is a quality reserved solely for the divine. Likewise, we humans are all imperfect copies of our ideal selves. For starters, within the billions of characters of DNA code we inherit from our parents, we each have roughly 70 spontaneous, single-letter typos.

"There are mistakes all over our DNA," says Sally A. Camper, Ph.D., chair of the Department of Human Genetics at the University of Michigan Medical School. "The average person has dozens of tiny additions, deletions and changes in their genome ranging from those single base pairs up to several hundred kilobases — several hundred thousand base pairs."

The vast majority of these microscopic misspellings have no noticeable effect, like mortars falling harmlessly in the vast hinterlands of our genetic geography. But these errors can also strike vital targets in our genes and sow havoc, giving rise to mutations that can cause cancer and birth defects — not only in an individual, but also potentially in their children and future generations.

"Our department and others across the medical school have a lot of strength in the area of genomic instability research," says Camper, the James V. Neel Collegiate Professor of Human Genetics and professor of internal medicine. Focusing on questions central to how cells maintain and protect the integrity of the human genome, U-M researchers are shedding light on a number of areas including mistakes that occur when cells copy themselves, dysfunctions in the protective end caps of our chromosomes, and small, "jumping" genetic elements. This type of "discovery science" is grounded in the basic human need to understand and illuminate the workings of our world and our bodies, but it also aims to better predict genetic risk, detect disorders earlier, and could lead to the development of new therapies and treatments.

Research, for example, led by professor Thomas W. Glover, Ph.D., has shown that certain drugs can disrupt cell division and create copy number variants — a recently discovered type of error that arises frequently in cancer cells and that can lead to birth defects including autism, intellectual deficiency, and psychiatric disorders.

Yet the more scientists learn about the genetic underpinnings of disease, the clearer it becomes how much we still don't know. About 40 percent of the children admitted to the U-M's C.S. Mott Children's Hospital have underlying genetic defects. To-date there are about 3,000 human disorders with a known genetic basis — and 4,000 candidates whose precise origins remain a mystery. "It's a really exciting time to be a geneticist," says Camper.

THINK of the genome as an encyclopedia containing the full blueprint for the human animal. It's divided into 23 volumes — chromosomes — written using only four characters: molecules abbreviated A, C, G and T. Each tome, in turn, contains sequences of coded instructions — genes — for the proteins behind thousands of different human traits, from eye color to blood type.

With increasing clarity, however, researchers at the U-M and around the world are coming to understand that this text is far less stable than was thought even a few years ago. Advances in technology are giving scientists' explorations revolutionary breadth and depth. Some of the larger collaborations, like the 1000 Genomes Project, involve hundreds of researchers across the globe.

The vast majority of these **MICROSCOPIC MISSPELLINGS** have no noticeable effect, like mortars falling harmlessly in the vast hinterlands of our genetic geography. But these errors can also strike vital targets in our genes and sow havoc.

"The reason for the many collaborators is that in the past couple of years the technology has changed such that we can go and sequence entire genomes. This gives us an avalanche of information that we didn't have before," says assistant professor Jeffrey M. Kidd, Ph.D., who was part of the 1000 Genomes Project and several other large-scale endeavors. "It takes a lot of effort to just acquire the data and even more effort to make sense of it. In practice this means lots of work on the UNIX command line, lots of hard drives mailed around the world and lots of conference calls."

Last year, Kidd was the first author of a paper published in the *American Journal of Human Genetics* that showed people with different ancestries have different degrees of variation

PREVIOUS PAGE: ILLUSTRATION BY ANN ELLIOT CUTTING


Sally Camper


Jeffrey Kidd

within their genomes, with African genomes being the most diverse. The rest of humanity is more homogenous because everyone else can be traced back to a small population that migrated off the continent some 60,000 years ago. The study also found, surprisingly, that while the proportion of similarity and difference within the genetic code of people of the same ancestry is consistent, it differs dramatically across populations.

"The big questions we're trying to answer involve understanding how genomes change both within a species as well as looking for variation across species," Kidd says. "There are specific regions of the genome that we know are prone to undergo the type of rearrangements that affect human health. We're working to understand why it happens there — is it just by happenstance or is there some sort of deeper long-term evolutionary process? My focus is to ask questions mostly over evolutionary time, but some of these variations are giving rise to disease right now, which is the side of the coin that faculty in our department are working on."

GLOVER says there are two reasons the topic of genomic instability should matter to everyone: cancer and birth defects.

"It's important to your kids and your grandkids and to you," he says. "With any kind of mutation there are going to be environmental and genetic factors that increase risk. If we can identify those on the environmental side we can avoid them. As for the genetic factors, they're important for understanding reproductive and disease risk. That's the potential payoff to all of this."

Just as takeoff and landing are the most dangerous times for airline passengers, in our DNA, when things go awry, they often happen when a cell is making a copy of its genetic material as it divides to form a new cell. Until the advent of new genomic technologies and next-generation DNA sequencing, chromosome abnormalities were detected under the microscope using a process known as karyotyping. Looking through the eyepiece, scientists were able to see big anomalies, like whether there was an extra or missing chromosome — a condition known as aneuploidy, the most well-known result of which is Down syndrome — or large structural changes within chromosomes.

For decades Glover has been studying "fragile sites," hotspots where DNA has a tendency to break when a cell is under stress, or dividing rapidly, as in cancer. Lately, he has also become immersed in the newly discovered riddle of copy number variants (CNVs), which are tiny additions and deletions to the genome too small to see using the old techniques, though they can span many genes. They arise frequently in cancerous cells and de novo CNVs — new variations seen in a child but not in her parents — are also believed to be responsible for 10 to 15 percent of developmental disorders.

"We now know that these deletions and duplications that are too small to see with a light microscope are surprisingly common across populations and arise frequently by new mutation — and we didn't even know they existed until a few years ago," says Glover.

But it was when Glover and his colleagues decided to use new technologies to examine the fragile site phenomenon that they made a big discovery. The team grew a colony of copies from a single human cell that was treated with a drug known to induce fragile sites by stressing the cell and disturbing its replication. At that higher resolution, they saw lots of

LEFT: ERIC BRONSON, MICHIGAN PHOTOGARPY
RIGHT: SCOTT SODERBERG, MICHIGAN PHOTOGRAPHY

new little deletions and duplications not only at the fragile sites but all across the genome — about three per cell.

"I immediately realized that they were identical to a major type of CNV," recalls Glover. "And we found that if you perturb replication you can recreate them. This suggests our DNA replication processes are frequently being disturbed and redirected in ways we don't yet fully understand.

"Yet, despite their importance, we know very little about the mechanisms that give rise to most CNVs and almost nothing about their risk factors," he adds. "So that is the focus of our research."

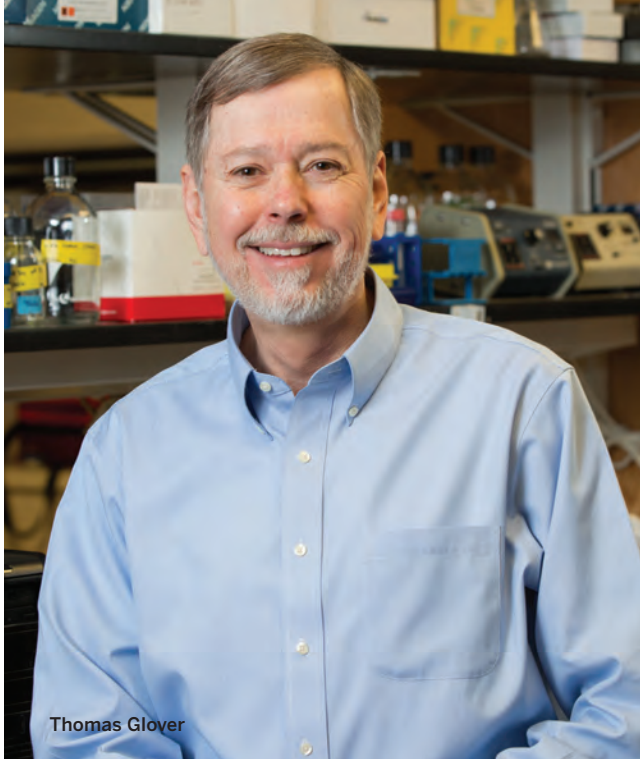
The search for answers was kick-started by a 2009 stimulus grant from the National Institute of Environmental Health Sciences. One goal was to see whether hydroxyurea, a replication-inhibiting drug used to treat patients with sickle cell anemia and some cancers, similarly induced CNVs in cell cultures. It did.

"We found that hydroxyurea a creates the same genetic signatures that we see in a large number of human patients with disorders caused by CNVs," says Glover, who worked closely on the project with Thomas E. Wilson, M.D., Ph.D., associate professor of pathology and human genetics, and human genetics research investigator Martin Arlt, Ph.D.

There are three ways in which such mutations could be harmful to people. First, they might cause dividing body cells to become cancerous; though hydroxyurea has been used to treat patients for decades and studies so far have not found an increased risk of cancer, Glover notes. Second, mutations occurring during fetal development might cause birth defects if the drug is taken during pregnancy, a known risk with hydroxyurea. Third, they might results in what are called germline mutations, a mutation in a cell that will ultimately become a sperm or egg. For men, mutations in cells leading to sperm could lead to birth defects in his children. However, all the eggs a woman will ever have are primarily formed before birth — so if a pregnant woman is exposed to an agent that causes CNV mutations in the cells that form the eggs of her unborn daughter, those effects wouldn't become evident until the daughter had children of her own.

"In other words, they would show up in the grandchildren of the exposed female," says Glover. He currently has a new grant from the NIEHS to look at such generational effects from hydroxyurea in mice — a slow and expensive process, but one which could have major health implications for millions being treated for sickle cell disease worldwide.

"While hydroxyurea is an effective and highly beneficial drug for many thousands of people, it is important to understand any potential risks to future generations," he says. It's also important, he notes, for scientists to start identifying other drugs and environmental contaminants that present the greatest risk for CNV mutations.


Thomas Glover

SCOTT SODERBERG, MICHIGAN PHOTOGRAPHY

COPY number variants aren't the only genomic wrinkle U-M geneticists are investigating. John V. Moran, Ph.D., the Gilbert S. Omenn Collegiate Professor of Human Genetics and professor of internal medicine, is studying a type of genetic element capable of "jumping" — that is, copying-and-pasting itself into far-flung regions of the genome. As a class of transposable elements, LINE-1s (Long INterspersed Element-1s, or L1s for short) make up about 17 percent of the human genome, but only a tiny subset are still capable of moving.

"It had long been thought that L1s were molecular fossils, that they were incapable of moving to new locations," Moran says. "That view changed decidedly in 1988 when my postdoctoral mentor, Dr. Haig Kazazian, discovered mutagenic L1 insertions in two different patients with hemophilia A, disrupting the factor VIII gene which is very important for blood clotting. These data showed that L1s are still capable of jumping to new genomic locations and have the potential to mutate genes, leading to human disease."

Twenty-five years later, L1-mediated jumping events have been estimated to be involved in roughly 1 in every 250 disease-causing mutations. Along with hemophilia, the phenomenon has been linked to Duchenne muscular dystrophy and colon cancer.

Moran's lab is delving into how L1s move to new genomic locations, what impacts they have on the human genome


John Moran


Catherine Keegan

and gene expression, and what built-in defense mechanisms may have evolved to suppress such an inherently destabilizing process.

"There's probably an evolutionary arms race going on between the host and the transposable element, which left to its own devices, might jump as much as it can," Moran adds.

Meanwhile, Catherine Keegan (M.D. and Ph.D. 1996), associate professor of pediatrics and human genetics, is studying the role telomere disorders may play in birth defects. Telomeres are repetitive sections of DNA that cap the end of each chromosome and help protect it — similar to a plastic aglet at the tip of a shoelace.

"We're really just starting to learn about how telomeres are important in a condition called dyskeratosis congenita, an inherited bone marrow failure syndrome," says Keegan, whose doctoral research was done in Camper's lab. "We're working with a mouse model that has a defective telomere protein.

"On some strains the mice with the mutation live and have features that resemble dyskeratosis congenita — including poor growth, abnormal skin pigmentation, and defects in the function of hematopoietic stem cells, which give rise to blood and immune cells. On other strains, the mice have congenital deformities of the lower spine that mimic a condition known as caudal regression syndrome and don't survive past birth. We've been interested in figuring out how this defect in the telomere protein causes the defects that we see in the mice."

New understandings gleaned in the laboratory are starting to have meaning on the clinical side as well, says Keegan. Investigation of rare genetic mutations can provide a valuable opportunity both to better understand complex biological processes and to help families get answers and end their "diagnostic odysseys."

"I was just at a grant review meeting where there was a proposal to study a rare disease and someone brought up, 'Well this isn't exactly a public health concern,'" Keegan says. "But that's not the point. The point is that we learn so much from these patients. It's really fascinating that we can say, 'Now we know that this particular gene causes this set of features in a child, and we can use that information to better understand the underlying developmental pathways.'

"Having an answer actually is helpful to the family," Keegan continues. "If we can find an explanation, we can help them work through the risks for future pregnancies. There also may be other known medical problems that child is at risk for that we can then help to head off. Even if there's no available treatment, I think many parents feel like, 'OK now I can move on from trying to find the answer and focus on doing what I can do to help my child.'" [M]

Investigation of **RARE GENETIC MUTATIONS** can provide a valuable opportunity both to better understand complex biological processes and to help families get answers and end their "diagnostic odysseys."

Read more about the department's research into cancer, birth defects and other topics at medatmich.org/HumGen.

LEFT: FILE PHOTO
RIGHT: AUSTIN THOMASON, MICHIGAN PHOTOGRAPHY