Dorcas Cummings Lecture

David Page

Dr. David Page presented the Dorcas Cummings lecture entitled "Sex and Disease: Do Males and Females Read Their Genomes Differently" to friends and neighbors of Cold Spring Harbor Laboratory and Symposium participants on Saturday, June 3, 2017. Dr. Page is a Professor of Biology at the Massachusetts Institute of Technology, an Investigator of the Howard Hughes Medical Institute, and the Director of the Whitehead Institute for Biomedical Research.

Thank you very much for the invitation to speak and for your generous introduction. I would like to thank all of you for your presence here this evening. It is an honor and a privilege to speak at Cold Spring Harbor Laboratory on any occasion and especially in memory of Dorcas Cummings.

I invite you now to join me on a journey from the past to the future with my favorite chromosome. Actually, with my favorite pair of chromosomes. On the left, the X chromosome: proud, statuesque, respectable. On the right, with its head down, the Y chromosome: diminutive, demure, downtrodden. Truth be told, I have spent my entire career defending the honor of the Y chromosome in the face of innumerable insults to its character and its future prospects. I ask you, men and women of the Cold Spring Harbor community, how could the Y chromosome get such a bad rap?

To understand the tragic past of the Y chromosome we've got to go back more than a hundred years, to 1904, when Charles Benedict Davenport became director of this laboratory. Just a few years later, he would establish Cold Spring Harbor as a leader in human genetics, then framed as eugenics, when he founded the Eugenics Records Office here. During those opening years of the 20th century, the principles of inheritance deduced in the 1860s by Mendel in his garden of peas were rediscovered and rose to prominence. In quick succession, three great modes of inheritance were reported in our species: autosomal recessive, autosomal dominant, and X-linked recessive.

It turns out that in a paper from 1907 several investigators claimed a fourth mode of inheritance: Y-linked inheritance. This report [Tomassi, *Arch Psichaitr Neuropat Antropol Crim Med Leg* **28:** 60 (1907)], which I'm sure that Charles Davenport read with considerable interest, was published in the *Archives of Psychiatry, Neuropathology, Anthropology, Criminology, Medicine, and Law*. This was one of the early interdisciplinary journals (you thought *Nature* was broad). The trait under consideration was "hairy ears," big tufts of hair growing from the earlobe, and the argument for its Y-linked inheritance looked pretty decent, with father-to-son transmission across the family tree—maybe a few guys in the last generation shaved their ears—but otherwise, it looked quite promising. Over the ensuing 50 years, a number of other traits were also claimed to show Y-linked inheritance. The first half of the 20th century was a heady time for the Y chromosome.

But the good times for the Y chromosome came to a crashing halt in 1957 in Ann Arbor, Michigan, at the annual meeting of the American Society of Human Genetics. There, the society's president, Curt Stern—who actually was a *Drosophila* geneticist from the University of California at Berkeley—delivered a colorful presidential address that was entitled "On Porcupine Skin and Hairy Ears or, The Alleged Sins of the Y Chromosome," although the editor of the society's journal cleaned up the title prior to publication to the more pedestrian "The Problem of Complete Y-Linkage in Man" [Stern, *Am J Hum Genet* **9:** 147 (1957)].

In his presidential address, in front of all the human geneticists of North America, Stern cataloged and debunked "all seventeen presumably or possibly Y-linked traits," including porcupine skin and hairy ears, showing all of them to be flimsy claims that were based on shoddy pedigree analysis. By the end of Curt Stern's presidential address, no genes were left standing on the Y chromosome. The best that Stern could do to cheer up the chromosome was to suggest that since it exists, it must have a function, concluding, "That the Y chromosome has a function of its own is attested by its very existence. What it is still must be discovered." Actually, Stern's scholarly debunking was absolutely right; none of the previous claims of Y-linked genes withstood scrutiny. It was not Stern's intention, but his defrocking of these spurious claims led others to a new understanding of the Y chromosome: It must be a genetic wasteland.

This was not the low point for the Y chromosome. It would get much worse, and our lab was partly to blame. In the 1990s, one of my graduate students, Bruce Lahn—now a professor at the University of Chicago—showed that our X and Y chromosomes had evolved from an

Published by Cold Spring Harbor Laboratory Press; doi: 10.1101/sqb.2017.82.035550

ordinary pair of chromosomes (autosomes), which had been identical in males and females of our reptilian ancestors. Subsequent work in my lab confirmed what was feared: Over evolutionary time the X chromosome had done a superb job of nurturing and preserving the genes of the ancestral autosome, while the Y chromosome had callously and carelessly frittered them away.

It turns out that 300 million years ago, when we were reptiles, we had no sex chromosomes. We had only ordinary chromosomes, and they came in pairs. One such pair of ordinary chromosomes did not know it, but they would evolve to become our X and Y chromosomes. They were a happy pair: They engaged in free trade, they swapped information, all sorts of stuff. Then something happened: One member of the pair sustained a mutation, giving rise to the sex-determining gene on what would become the Y chromosome. The two chromosomes had formerly been in close communication with each other, but the nascent Y chromosome then changed its behavior. It said, "Enough of this. I am going to adopt isolationist strategies." The Y chromosome decided to go its own way. These isolationist strategies of the Y chromosome led, not surprisingly, to the decline of its economy. The Y chromosome lost many of its genes, becoming a shadow of its former self. Simultaneously, the X chromosome expanded enormously, at the expense of the Y chromosome. So we ended up with a much smaller Y chromosome containing the sex-determining gene, overshadowed by the giant X chromosome.

In 2002, two colleagues in the field saw an opportunity to deal a truly fatal blow to the Y chromosome and published a punishing editorial in a weekly journal of some repute. In a *Nature* editorial grandly titled "The Future of Sex" [*Nature* **415:** 963 (2002)], John [Aitken] and Jenny [A. Marshall Graves], my good friends, concluded that "… the Y chromosome is particularly vulnerable … because it is not a matching partner for the X chromosome, so it cannot retrieve lost genetic information… ." After recounting the tale of the chromosome's diminishment that I have told you today, they delivered a devastating punchline: "At the present rate of decay, the Y chromosome will self-destruct in around 10 million years."

I had been planning to make a career out of the Y chromosome.

I was not the first in my lab to read this editorial. It was one of my graduate students, who came running into my office with tears streaming down his face. We held an emergency lab meeting, and we resolved to pick up the pace of our research.

We could not move quickly enough. A comic book series called *Y: The Last Man* burst onto the scene. The series consistently made subtle use of "Y" symbolism and inspired the production of a decidedly bad movie (*The Last Man on Planet Earth* [1999]), whose premise was that, "… feeling they were better off without males, the women of Earth decided to outlaw men because they were too violent. They developed a weapon called the Y-bomb, which resulted in the deaths of 97% of men." I tell you, no other chromosome has had to put up with such attacks. "Twenty years later, a scientist …"—and what else could she be called except Hope Chayse?—"… conducts a cloning experiment to produce a new male whom she names Adam. When Adam reaches maturity, he finds himself on the run, hiding out with rebel bands of the last remaining men." Actually, the last remaining men end up hiding in an abandoned NFL football stadium. I highly recommend this movie to you, if you can find a copy of it. Believe me, it is not available on any of the streaming services.

Anyway, it got worse. The Internet became littered with models of the Y chromosome like this one, with "genes" like the "channel surfing gene" (*FLP*), which sometimes is up here and then it flips down to here; the "balls, two" gene (*BLZ-2*), which confers self-confidence unlinked to ability; the *DC10* gene, which confers the ability to identify aircraft in the sky; and the *MOM-4U* gene, which drives young sons to present spiders and snakes to their mothers. Also included in this model of the Y chromosome is the well-known *P2E* ("ptui") gene (codes for spitting); and then one that my wife is convinced is closely linked to the inability to remember anniversaries and birthdates, the *HUH?* gene for selective hearing loss.

This is what I have had to deal with. Something had to be done to stop this public humiliation of the Y chromosome, so our lab responded with help from our sister species. Here I would like to tell you a tale of three primates, or at least their Y chromosomes. We turned to the rhesus monkey, a chimp named Clint, and, last but not least, a human. I thought this would be an appropriate time and place to identify the man whose Y chromosome we sequenced: none other than [Symposium organizer] Bruce [Stillman].

I don't want to drag you through the details of the DNA sequence analysis, but I had an opportunity to discuss our results on *The Colbert Report* [3/26/12; http://www.cc.com/video-clips/rc1xqe/the-colbert-report-david-page], where I summarized what we learned by comparing in detail the Y chromosomes of these three species. We found that the human Y chromosome and the rhesus Y chromosome carry essentially the same genes. This suggests that nothing much has happened to the Y chromosome in the last 25 million years. The Y chromosome was in a steep nosedive, losing genes at a furious pace, but then it leveled out and has been flying at a low but steady altitude since, so men are going to be okay.

Thus ends Part One of this lecture. Now, having rescued the Y chromosome from a century of misunderstanding, let me suggest that the Y chromosome, together with its partner the X chromosome, may play a critical role in the future of medicine.

What do we know today about the role of the Y chromosome in medicine? The Y chromosome is known to carry a single gene that causes a human embryo to develop testes rather than ovaries, and deletions of the Y chromosome's sperm production genes are the most common known genetic cause of male infertility in our species. However, what I want to tell you about today extends far beyond the reproductive tract and to diseases that occur in females as well as males. My topic is sex and disease (but not what you think). I would like to share with you what I think is the really important link between sex and disease, a connection that is not talked about enough.

Let me get the ball rolling by sharing with you three observations that might surprise you. First, I am going to suggest that our concept of the human genome is off the mark. Second, that males and females are not equal. Third, that the study of disease is flawed in significant ways.

With respect to my first point, there are times in history when scientists, as brilliant as they sometimes are, have gotten things wrong. For centuries many smart people thought the world was flat. We also thought the Sun revolved around the Earth. In this age of the genomic revolution, I am going to suggest that we are missing something vitally important.

Let's begin where we all began: a fertilized egg. All the cells in your body—your heart cells, your brain cells, even your skin cells—all derive from this one cell, the fertilized egg. This special cell divides to become two, four, eight, and so on until we reach the roughly 10 trillion cells that make up our body. What's amazing is that within the nucleus of each of your 10 trillion cells, you carry the same 23 pairs of chromosomes, which contain all of your DNA, all the instructions your body needs to function. There are 22 pairs that are the same in males and females, and then comes the 23rd pair, which in females is a nicely matched pair of X chromosomes. In males, that 23rd pair is a mismatched X and Y.

As I have told you, the Y chromosome has always been underestimated. Even today, most scientists and physicians think that the Y chromosome is important only within the cells of our reproductive tract. This erroneous assumption has led them to believe that, apart from the reproductive tract, the genomes of males and females are functionally equivalent. In fact, the Human Genome Project and the resultant recent initiatives in precision medicine are based on our being 99.9% the same at the genomic level. This idea has gained traction for many reasons. It sounds great politically to say that we are all 99.9% the same. In fact, Bill Clinton actually used this idea to bring the country together in his 2000 State of the Union speech: "This fall at the White House, we had this very distinguished scientist there, who is an expert in this whole work in the human genome." I will not name any names. "He said that we are all, regardless of race, genetically 99.9% the same."

This sounds great, and it is even true if the two individuals you are comparing are both males. It is also true if the two individuals you are comparing are both females. If you make a mistake, and you compare a male and a female, they are only 98.5% identical. Let's flip this around: Instead of talking about degree of identity, let's talk about the degree of difference. In other words, between two males, it is a 0.1% difference; between two females, a 0.1% difference. It is a 15 times greater difference in the genomes of male and female: 1.5%. What is that difference? Of course, it is XX versus XY.

What is called "precision medicine" today is really the study of the 0.1% genetic differences between two men or between two women. And we are now committing, quite appropriately, at the national level, hundreds of millions of dollars to this study of precision medicine. But by comparison, the 1.5% genetic difference between males and females has no name or federal program devoted to it, no banner or slogan. Let's call it "sex differences." The area of sex differences has barely begun to receive funding or the focused attention of dedicated researchers.

But how biologically or clinically significant are these sex differences—these genetic differences between males and females? It turns out that a male human is as closely related to a female human as he is to a male chimp: there is a 1.5% difference between male and female humans, just as there is a 1.5% difference between male and female chimpanzees. The human genetics revolution has missed this important fact. Our field has instead created a unisex model, when in fact males and females are not equal—they are not equal in their genomes, and they are not equal in the face of disease.

What do I mean by this and why does it matter? Let me give you a handful of examples. Take rheumatoid arthritis. For every man who has rheumatoid arthritis, there are two or three women with the disease. Is rheumatoid arthritis a disease of the reproductive tract? No. Is it anatomically obvious why women should suffer from this disease two to three times as frequently as men? No. There is no simple explanation to be found in our anatomy.

Let's flip it around: Autism spectrum disorder. The latest statistics suggest that for every girl that has an autism spectrum disorder, four boys are affected. Why is that the case? Let's flip it around again. Lupus. For every man who suffers with lupus, there are six women who suffer with the disease. And there are many other disorders that, like lupus or autism, are more common in females or in males. For other diseases where the incidence is similar in males and females, the severity or consequences of the disease may be greater in one sex than the other.

Let's examine the example of dilated cardiomyopathy to illustrate why sex differences matter in medicine. A specific genetic defect causes a thinning of the wall of the heart and a dangerous ballooning. If you look at the survival curves—the "death" curves, if you will—for women and men with this disease and the same underlying genetics (autosomal dominant genetics, for the scientists in the audience), men die at a much younger age, about 10 years earlier [Herman et al., *N Engl J Med* **366:** 619 (2012)]. Nobody knows why. When I query medical specialists, academics, and researchers about this disorder, or any of the others I have shown or any of dozens of others that I could mention, when I ask, "Why is it that one sex is more commonly or more severely affected than the other?" I almost always get the same response: "I don't have a clue."

This is in an age of precision medicine.

If I press harder, the answer that many physicians and scientists come up with is, "Maybe it's sex hormones." It turns out that the human genetics revolution has provided us researchers with powerful tools to ask why one man is at higher or lower risk than another man for a given disease, or why one woman is at greater or lesser risk than another woman. However, incredible as it may sound, we do not yet have a toolkit to ask why males as a group are at higher or lower risk than females as a group. This is a big, big question, but no one has a clue about the answer. But maybe the answer has been staring us in the face all along:

That is, the individuals who tend to get diseases such as autism and dilated cardiomyopathy are XY, and the individuals who tend to get diseases such as lupus and rheumatoid arthritis are XX. This is a fundamental difference, right? It is present in all our cells, but we in the scientific community have been operating for about 60 years on a faulty assumption: that the Y chromosome is functionally important only in the reproductive tract.

I am going to give you a one-slide crash course in how sex differentiation is taught in every medical school in the world. Instructors teach that being XX or XY is of direct biological consequence only in the nether regions, in the reproductive organs. According to this longstanding view, all nonreproductive differences between males and females, including differences in disease susceptibility, should be attributed to the sex hormones: the androgens and estrogens that are produced by the reproductive organs and circulate throughout the body.

In recent years, however, my research group at Whitehead Institute has discovered that the Y chromosome is actually operating throughout the entire body, as is the X chromosome. The cells of your heart, your pancreas, your brain, your skin—they know whether they contain XX or XY chromosomes. I want to hybridize the old sex differences model with a new one, which acknowledges how the X and Y chromosomes have roles throughout the body. Accepting this reality will lead to a far better way to study disease.

I go to my colleagues performing laboratory research at medical schools, universities, drug companies, and even at Whitehead Institute, and I ask scientists who are working with human cells, "Are you working with XX cells or XY cells?" The answer I most frequently get is, "I don't know." How could you figure things out if you do not know or have not thought to ask whether you are working with XX or XY cells? This means that, in many cases, the research that is being done to discover the underlying causes of diseases, or new treatments for them, is not taking into account this most fundamental of differences between males and females. This is why I suggested rather provocatively at the beginning of this talk that the study of disease is fundamentally flawed.

What can we do about this? How can we rethink the relationship between sex and disease? First, I believe that XX and XY cells may do their molecular business a bit differently from each other, throughout the body. Scientists around the world need to incorporate this distinction into their research for treatments and cures. At my lab at Whitehead Institute, we are already doing this, and we have preliminary evidence that the way proteins are made may be slightly different in XX and XY cells.

We need a better toolkit for scientists and drug developers to use, one that recognizes and includes this fundamental difference between male XY and female XX cells, tissues, organs, and bodies. If we take these steps—and I believe we can—we will arrive at an entirely new paradigm for treating disease. It will really matter whether a patient is a female or a male—and not just to physicians with deep understandings of the reproductive tract, not just to gynecologists and urologists; it will matter to cardiologists, to endocrinologists, to dermatologists. I anticipate that a full appreciation of the roles of the X and Y chromosomes will fundamentally change the way that you, your children, and your grandchildren will experience healthcare in the future.

This work is going to require the efforts of many scientists in many laboratories in many countries. It is just getting started. Let me introduce you to a few of the early adopters in my own lab who have joined the cause: Winston [Bellott], Jen [Hughes], and Helen [Skaletsky], who, with our colleagues at Washington University [Richard Wilson, Wes Warren, Tina Graves, Robert Fulton] and Baylor [Richard Gibbs, Donna Muzny, Shannon Dugan], pioneered the comparisons among the human, primate, and other mammalian Y chromosomes that brought many of these questions to the fore. Lukáš Chmátal, a postdoctoral fellow whose previous work on centromere strength with Mike Lampson and Richard Schultz at Penn has been described by several speakers at this meeting. Lukáš is now examining sexual dimorphism in the human heart. Emily Jackson is a recently arrived grad student who, in her pre–grad-school life, trained with David Pellman, whose summary will close this scientific meeting.

Let me offer a glimpse of the directions in which my lab is taking this work. We are examining the roles of microRNAs in these processes; Sahin Naqvi, a graduate student, has found that conserved microRNA targeting reveals preexisting heterogeneities in gene dosage sensitivity that shaped sex chromosome evolution in mammals and birds [Naqvi et al., *Genome Res* **28:** 47 (2018)]. Working with 12 different tissues from five species, Sahin is beginning to scope out sex differences in gene expression across the body. I mentioned Lukáš' work on sex differences in the human heart [with Jon and Christine Seidman and Rick Mitchell at Brigham and Women's Hospital; Steve Gygi at Harvard Medical School]. We're examining the brains of mice and humans (looking at microglia in particular) [with Richard Ransohoff at Biogen; Chris Glass at the University of California–San Diego]; postdoctoral fellow Laura Blanton is exploring sex differences in immune cells in both mice and humans [with Dan Kastner at NIH; Andrew Lane at the Dana–Farber Cancer Institute]; and postdoctoral fellow Adrianna San Roman is studying those not-so-rare individuals who carry not two sex chromosomes, but one (XO), three (XXY, XYY, XXX), four, or even five (XXXXY, XYYYY) sex chromosomes, and their effects on global gene expression [with Max Muenke at NIH; Carole Samango–Sprouse at Focus Foundation].

In closing, whether you are a clinician or a lab researcher or a supporter of biomedical investigation, I want to challenge you to thoughtfully consider the approach that I have presented today, and how the knowledge of genetically based sex differences can transform our understanding of human health and disease. Thank you very much.