[ẩnannoncer:] From the Howard Hughes Medical Institute, the 2013 Holiday Lectures on Science. This year’s lectures, “Medicine in the Genomic Era,” will be given by Dr. Charles Sawyers, Howard Hughes Medical Institute investigator at Memorial Sloan-Kettering Cancer Center, and by Dr. Christopher Walsh, Howard Hughes Medical Institute investigator at Boston Children’s Hospital. The first lecture is titled “Sizing Up the Brain, Gene by Gene.” And now, a brief video to introduce our lecturer, Dr. Christopher Walsh.

[music plays]

[dr. walsh:] Our lab is committed to try to understand the genes that are essential to put the human brain together. And that’s a biological problem: just how do you get the right number of cells, how do those cells know what they’re supposed to do, how do they get put in the right place, and then how do they function properly. How do all those cells in our brain get wired up to generate the tremendous things that a human brain is capable of? Our work also has a medical component, because the genes that are required to put the human brain together, if they don’t work properly, they cause disease. And so our work focuses on working with families, and kids with neurological disorders such as intellectual disability, autism spectrum disorders, and epilepsy, and trying to understand what’s gone wrong in the brains of those kids. Our long-term goal in our work is to try to understand mechanisms of disease so that we can try to fashion better treatments.

We’re also studying what I think is one of the most exciting biological problems out there, which is trying to understand what is it that makes the human brain tick; what are the essential things that have to happen in order to enable things like intelligence and aesthetic ability and consciousness in humans. And we’re hoping that by trying to understand the many ways in which things can go wrong, we’ll get a better understanding of how it happens so reliably in such a large proportion of people.

By participating in the Holiday Lectures, I hope to help high school students get a little bit of a better understanding of how science really works. I want to try to help every high school student that might be a great scientist to find the right path.

[applause]

[dr. walsh:] Good morning, everybody. I’m Chris Walsh. I’d like to add my welcome to all of you at the Howard Hughes Medical Institute. I want to thank Dennis and Sean and Bob Tjian for giving me the opportunity to participate today. I’m really thrilled and honored to be here along with Charles Sawyers and Jim Watson to celebrate the 60th anniversary of the discovery of DNA. And I think it’s particularly meaningful for Dr. Sawyers and for me because we both have teenage kids just like you all, so this is a really important event for our families. I don’t mind telling you I’m just a little bit intimidated to have Dr. Watson, who discovered the structure of DNA 60 years ago, right here listening to me. But it’s a great thrill. And so over the next four lectures over the next two days, Dr. Sawyers and I want to tell you a few stories about how the recent explosion in our understanding of the genome has enabled us to do things in medicine and in science that we couldn’t have thought of even just a few years ago. I’ll
tell you a few stories about how we’re learning new things about how the brain develops and some of the disorders that affect the developing brain. And Charles will tell you a few stories about how the genomic revolution has enabled us to develop remarkable new treatments for cancer.

Now, at first thought, cancer and the brain don’t really seem to have that much in common with each other. I mean, after all, you know, the brain is the seat of our consciousness. It’s the apex of mammalian evolution. And cancer, on the other hand, undergoes its own sort of cellular, malignant evolution, where … as Charles will tell you about, where it’s also constantly changing to form more lethal and elusive forms.

But what we’ve learned over the last few years is that many of the same genes actually are used in remarkably similar ways, even in cells that form cancer that are constantly dividing, often out of control, and in the brain, where cells are not dividing, but are doing other things: communicating using electrical signals. And so the genomic revolution has really taught us, then, that not only technologies, but even specific gene names, cut across these different fields. And so that’s really what we’re here to celebrate is this genomic revolution that allows us to sequence the genome of a person in just a few days or a week, whereas as I say, it took us 20 years and $3 billion to sequence the first human genome.

Now, I think that most of you know that the human genetic code is written in an alphabet containing four letters—A’s and C’s and T’s and G’s, organized in these Watson-Crick base structures that were described 60 years ago. But what’s happened lately is that the technologies for sequencing the human genome have become very, very fast and very, very cheap. And this charts that trend over the last dozen years or so. This shows the cost of sequencing a million base pairs of DNA on actually an exponential scale, so you see it goes down by a factor of … the y axis goes down by factors of 10. You can see that DNA sequencing got cheaper and cheaper every year, but then around 2008, the curve just really started plummeting. And this was due to the introduction of new technologies which are generally referred to as next-generation sequencing technologies.

And so this has allowed us, then, to go from a cost of a $3 billion genome and 20 years to do it, to now being able to sequence a genome for 1 million times less money, only $3,000 as of the last time I checked last week. And it may be cheaper next week because the costs continue to drop. And you can sequence the exome, which is the parts of the genome that actually code for proteins, which is then just a couple of percent of the genome, for even less money than that. And so the availability of this large-scale genomics has allowed us to have new insights into human physiology and also even into tracing evolutionary relationships, as well as improving medical therapies. And there will be a couple of points in my own talks as well as in Charles’ talks about these areas.

First I just want to tell you a little bit about the brain, give you a short introduction to its structure and how it functions. It’s a big topic—a little bit too big to cover in just a few minutes. But I just want to give you a short introduction. And so I’ll use these models over here. The brain is an undistinguished looking structure. It weighs about three pounds, you know, about a kilogram and a half. It has these
prominent folds on the outside. The part I’ll be talking about today is the cerebral cortex, which is the folded part on the surface of the brain, and it makes up about 80% of the volume of the whole brain.

Now, if we section it sort of like this—and I’ll be showing you a lot of pictures of the brain sectioned like this, as illustrated here, because that’s how we tend to do magnetic resonance imaging to image the human brain while people are alive. Anyway, if you section the brain like that, you see something like this, where the cortex itself—“cortex” actually means “lining”—and it’s actually the gray matter around the outside here. And this is where the actual brain cells live. They’re called neurons. They have gorgeous structure that look a little bit like trees, as illustrated by the picture on the right back there. They have branches which are called dendrites that receive information from other neurons. They have a cell body where the nucleus lives. And then they have a single long process that’s sort of like a stem, that branches. And then that projects to other neurons and passes information on to other neurons. So the cell bodies live in this cortex. And then actually, those axons, those fibers that connect one neuron to another, run out from the cortex down and connect other sorts of structures. And this white area, called white matter, is where those fibers are located. And it actually looks white because those fibers are coated with a fatty coating called myelin which acts like an insulation. Just the way insulated wires are insulated, so are the fibers in the brain insulated as well.

Now, we can see even more beautiful ways of looking at the brain with newer technologies, and that’s shown here. There’s an amazing technology that’s just been developed in the last couple of years where you can label some of the neurons in a brain. This is a mouse brain. And you can use fluorescent proteins that make them glow. And then you can actually section the brain into thick pieces, but then make the brain clear by a special treatment using acrylamide that stabilizes the cellular proteins and then dissolving away some of these fatty lipids so that we can visualize the cells inside the brain a bit more clearly.

And if we can run the video, we can see what that looks like, where it gives you an ability to really see inside the brain and see what these neurons look like. The cortex is the outermost structure at the top of the picture, and there are several of the neurons that are labeled up there. And there are other neurons in the deeper brain structures which form that C shape in the middle of the picture. And you can see some of those dendrites that are outlined with the fluorescent marker radiating out from the top of the cortex.

So the brain is all about connections between cells. It’s about processing information. And the connections of one neuron to another are very specialized, and they’re called synapses. And I’ll have more to say about synapses tomorrow.

So putting a brain together, getting all of those neurons in the right place, making all of those dendrites and axons, making all of those connections, you might imagine is a pretty complicated deal to get that to actually work. It requires a lot of genes. We have 20,000 genes in our genome, and the brain probably uses at least half of them. And so you can imagine, then, that unfortunately, nature doesn’t always get it right, and that there are a lot of things that can go wrong with the development of the
brain. Would anyone here like to volunteer any thoughts about developmental disorders that might affect the human brain that you might have heard about? Yes?

[STUDENT:] There’s Alzheimer’s, where it’s a buildup of a really complex lipid in the brain.

[DR. WALSH:] Alzheimer’s, that’s a very good thought. Alzheimer’s is more of a problem of adult brains, where the brain gets put together good, but then actually tends to sort of fall apart or degenerate, so the neurons are lost. In the back there.

[STUDENT:] Epilepsy?

[DR. WALSH:] Yes. Epilepsy’s a really good example. It often affects kids. And that is a condition where the electrical activity of the brain is not under the proper control. Do you have an idea?

[STUDENT:] Down’s syndrome?

[DR. WALSH:] Yes, exactly. That’s a great thought. That’s a great one, where you have an extra copy of an entire chromosome. And the neurons actually seem to be present in about the right numbers, but don’t seem to work quite properly, probably because the synapses are weak.

There are also ... a little less well known are a whole host of structural disorders of the brain, where in fact the neurons don’t get put in the right place. They are formed in not enough numbers, and we can actually see these structural disorders very clearly now because we have such tremendous ways of imaging the structure of the brain itself. So this shows you an example: on the left is a section of a brain of a person who died, and they actually took microscopic sections of that brain. You can see again the purple cortex around the outer lining and the white matter underneath. But in fact, you can get pictures that are almost as clear and almost as sharp by using MRI imaging, as you can see over on the right. The picture there actually shows you the gray matter as gray and the white matter as white. And it has almost the same resolution as the microscopic image on the left. And so MRI imaging has allowed us to see a tremendous amount about the structure of the human brain and structural disorders of the human brain as well. And here’s an example of what some of those disorders can look like in the MRI scanner. You can see that normal image on the left, and perhaps you can see some of the ways in which the brain can be disrupted. And these each represent a disorder of a single gene out of the 20,000 genes in our genome that disrupt the brain in very distinctive ways.

For example, you can see some of the brains look too small. That’s a condition called microcephaly, which just is a fancy, unpronounceable word for “small brain.” And I’ll have more to say about that in the second half of this morning’s lecture. There are other disorders where the brain looks to be about the right size, but is the wrong shape because it lacks the normal folding pattern that distinguishes the normal brain. And also, here’s a condition where the brain doesn’t develop properly. And it’s shown again in MRI imaging. There’s an image of a normal brain on the left, and then this abnormal brain is shown on the right panel. Anyone want to hazard a guess as to what you can see that’s abnormal about the image on the right? Yeah.

[STUDENT:] It’s not symmetrical.
[DR. WALSH:] Exactly right. Any other thoughts? Yeah.

[STUDENT:] One hemisphere looks significantly larger than the other one.

[DR. WALSH:] Exactly right. And that’s really the fundamental issue that’s a problem with this child. And so this is a child who, right from the day of birth, started having uncontrollable seizures. And do you want to guess which of those two sides of the brain the seizures were coming from? Is it the big side or the little side? Yeah.

[STUDENT:] The big side.

[DR. WALSH:] Exactly. They are coming actually from the big side because the big side didn’t develop properly. And actually, you might think having a bigger brain is good for you, but the particular way in which this brain developed to become too big was very, very bad for this child. Right from the day of birth, the seizures started. And they came in waves. And they were happening 100 seizures or more a day. And that part of the brain, that right side of the brain, didn’t work properly. So it wasn’t doing what it was supposed to be doing, and it was doing lots of things that it wasn’t supposed to be doing. So the left side of the body, which is controlled by the right side of our brain, for reasons, again, that are sort of complicated. The right side of the brain wasn’t working properly, so this child had weakness of the left side of the body. The right side of the body seemed to work fine because the left hemisphere was good. But that left side of his body controlled by that right brain was not working properly at all. And so this is a disorder that’s called megalencephaly, which means, “large brain.” It’s the opposite of microcephaly. But it’s called hemimegalencephaly, because only half of the brain is abnormally large.

And as I said, it’s not only abnormally large, but it’s poorly developed. Perhaps you can also appreciate that in that large right brain, the white matter and the gray matter look sort of blurry. The white matter looks sort of gray, and the gray matter looks a little bit paler gray than normal. And that’s because, in fact, if you looked under a microscope, the cells are not normal either. The glia, the glial cells that make up the white matter don’t look like normal glial cells, and the neurons that make up the gray matter don’t look quite right either.

And so this child had a huge problem. He could not show any cognitive advancement. He couldn’t learn to walk. He couldn’t learn to talk. He couldn’t do anything because he was having seizures essentially all the time. And no matter what the doctors tried to treat those seizures, they couldn’t stop them. And so he finally underwent a surgical procedure which sounds incredibly radical, but in his case, was tremendously beneficial. They actually removed half of his entire cerebral cortex. So this is a procedure called hemispherectomy because the two halves of the brain are called hemispheres. And you can see that that right half of the brain was removed, and now it looks all white because that’s actually water. That’s what water looks like in the MRI scanner with this particular technique. And so there’s no brain there anymore. Now, this child has only half of the normal cerebral cortex.

And you might think, why in the world would you ever even think about doing something like that to a child. You might imagine, ... you might think about, you know, what does this child look like now where
he has only half a brain. But in fact, this procedure essentially stopped his seizures and allowed him to develop. And we have now a video to introduce you to that boy, whose name is Dante, to show you exactly what he does look like. And so he has had one hemisphere of his brain removed, and we can run the video now, and the family can tell us about it.

[FATHER:] So Dante is a 10-year-old boy. And although the right side of his brain’s removed, he still acts like a 10-year-old boy, which is awesome.

[MOTHER:] We’re talking about you.

[FATHER:] We’re talking about you. But I mean, obviously, because he has the right side of his brain removed, the left side of his body is weaker and is not able to do a lot of the fine motor skills, you know, that his right hand can do. But, I mean, he goes to school. He can read. He talks. You know, he loves, … he’s been riding horses since he was 1 year old. And he loves bowling. His major love is bowling. You know, we’re bowling all the time. We got each other our own bowling balls and bowling shoes. And it’s something that he can do because you really only need the right side of your body to do it.

[MOTHER:] You know, he’s acting silly right now, but this is how he acts all the time. I mean, he could be in the hospital for four months at a time, and he has this silly attitude, and he just wants to read, and he wants to be happy, and he wants to make others proud. And so I think he has a drive within him that has helped him come so far, because, you know, even in the worst of times when, maybe the seizures have returned, and we’re back in the hospital, and the neurosurgeons come along, and they really don’t have any answers for him, they’ll say, “Well, wow; I’m just really impressed with who he’s become. You know, I never really thought he’d be who he is today.” And he’s just—he likes to prove everybody wrong. And I love that about him.

[DR. WALSH:] So I just want to thank Dante’s family for appearing on the video. Dante’s mom’s a high school teacher herself, so she also shares our commitment to trying to get people inspired about science and to try to help build the next generation of scientists. So you can see that Dante’s not normal. But he can talk, he can read. He goes to a special school. You can see he’s particularly weak—his left arm is the weakest part. But that right hemisphere wasn’t working anyway, and so that’s no worse than he would have been if that abnormal brain had been left in there. And everything else is much better.

So what causes hemimegalencephaly? What causes one side of the brain to get bigger than the other side, to have the brain develop asymmetrically like this and for it to lose sight of what its normal size is supposed to be? So this is something that happened before birth. Anyone have any thoughts about what might cause a part of the brain to get to be too large? Yeah.

[STUDENT:] One of the genes regulating the structure of the brain either had like a mis- … like it had one of the substitutions or deletions or insertions.

[DR. WALSH:] Right. Any other thoughts? That’s a good thought. Yeah.
[STUDENT:] A mutation in the gene that promoted mitosis on that side of the brain.

[DR. WALSH:] That’s right. Those are really good ideas, and that’s exactly the way our group and other groups who have studied this condition were thinking about it. The thing about it is, that we thought it must have been a mutation that occurred not in the germ line, not at the time of fertilization or not in the sperm or the egg, but sometime after that. So somehow, the mutation-containing cells ended up on one side of the brain and not on the other side of the brain. So this might have been a mutation that arose in the somatic cells. We call it a somatic mutation, during mitosis of the brain, but after the cells of the brain have become separated from cells in the other parts of the body.

The way to find the cause of this condition is to take an approach similar to the way Dr. Sawyers will tell you about how we try to understand cancer: by studying the abnormal tissue itself. And this can only be done in a small number of patients who have undergone this kind of radical surgery. And so to try to find the cause of hemimegalencephaly, we studied eight patients that had had this same surgery. The MRI on the bottom shows another patient—not Dante, but a different patient—that had a similar sort of overgrowth of the right hemisphere. And two of those first eight patients that we studied had the same remarkable abnormality of the chromosomes that we could see by measuring the amount of DNA from the various chromosomes in the tissue of the brain itself. And we could see that chromosome 1 had this bizarre change, where the long arm of chromosome 1, which is shown on the right of the screen, was essentially duplicated. There are two copies of the entire long arm of chromosome 1, which actually has about 1,000 genes in it. And so this made us think, maybe there is a gene on that long arm of chromosome 1 that controls cell division. And maybe if you have too much of this gene that promotes cell division, it might cause the cells in the brain to divide in a fashion which is abnormal and to divide too many times, and also to maybe prevent the cells from differentiating and maturing the way they’re supposed to.

Now, at the same time, we found other patients that had a deletion of a part of chromosome 1, so they were missing one of the normal copies. So instead of the normal two copies of the long arm of chromosome 1, they had two copies from most of the long arm of chromosome 1, but at the very end, near the telomere, which is the end of the chromosome, there was a small deletion. So instead of two copies of the genes at the end of chromosome 1, they only had one copy. And anybody have any thoughts about if this is the same gene involved, what that might do to the brain? Yeah.

[STUDENT:] One side of the brain’s going to be really, really large, and the other side’s going to be much, much smaller because that gene was deleted. So it’s going to way overcompensate.

[DR. WALSH:] Yeah. So we were thinking that maybe if you lost this part of the brain, it might end up being smaller, and that’s actually what happened. So that maybe there’s some gene that if you have too many copies of it, it makes your brain too big. But you don’t have too many copies of it in the whole brain, but only in the half of the brain in those patients where it’s too big. But if you’re missing it, if you have one copy instead of the normal two copies, maybe you don’t get enough push, enough oomph, and the brain ends up being smaller. And that actually is what happens. We defined patients who are missing one of the normal two copies, so they were down to a single copy, and their brain was
actually smaller. And this is really amazing to us to think that there might be a gene down there that controls the size of the brain in a dosage-sensitive way, where more of it makes it grow bigger, and not enough of it makes it grow smaller. It’s not usually that simple. And it’s probably not quite that simple. But it’s not far off.

And so actually, the patients with the deletion were very helpful, because they’re only deleting a small piece of the chromosome, and that allows us to zero in on what the specific genes might be. And by studying about a dozen different patients with the deletion—because this happens not uncommonly—we were able to localize the minimal genes that have to be deleted to make the brain small down to just about a half a dozen or so. And there was one of these genes that stood out as the most likely to control proliferation and cell size, and that’s a gene called AKT3, because in fact, this has been implicated as a cancer-related gene that controls the proliferation of cancer cells. And so we thought maybe it has a similar sort of effect, not to make cancer, but maybe to act in the stem cells to make too many cells.

But then somehow the neurons still manage to stop dividing, because neurons have a very strong push to stop dividing because they can’t really function in the brain as neurons unless they ultimately are post-mitotic. And so we considered this AKT3 gene to be the most likely gene to be affected. And we sequenced it in all of these eight patients, including Dante, the patient I introduced you to. And in fact, Dante himself has a different kind of mutation that affects just this one gene. So this shows part of the trace, the actual old-fashioned DNA sequencing trace with an older technology called Sanger sequencing. And that gives you these peaks in an electrophoresis. And you can see that the different colored peaks correspond to the different base pairs. There’s a T that shows up as a green peak, and then A that shows up as a red peak. G shows up as yellow. And if we sequence Dante’s blood, he has a completely normal sequence for this particular gene.

But when we sequenced just the tissue from Dante’s brain, we see something a little different. And can anybody see what the difference might be? Yeah.

[STUDENT:] There is an A in some tissue from his brain in the middle nucleotide.

[DR. WALSH:] Yeah. There’s just a little bit of a peak down there that looks like it’s an A. Instead of the normal G only, there is just this tiny little side peak. And we frankly would not have noticed this unless we were specifically looking for it. But that A actually corresponds to a mutation which is associated with certain cancers, and so it really caught our attention. And so I highlight that here where, as I say, it’s a very subtle change, ‘cause you can see that there is some background peaks. The technology is not perfect. There’s always a little bit of noise. But in fact, this is just a little bit higher than the noise. And it suggested to us that there might be a mosaic mutation or a somatic mutation: a mutation that’s present in some cells and not others because it occurred after the formation of the zygote, as the cells were undergoing mitosis, and that might be predominantly present in just that one half of the brain. So in this case, we’re thinking the mutation occurred late and is predominantly in brain cells and not in the other cells of the body.
And so what we actually did was to take that abnormal brain, and we separated cells one at a time, and we then sequenced this particular AKT3 gene in the DNA from individual cells, which again, is a remarkable thing that we’re even able to think about doing that. And we found that some of the cells in the brain were completely normal at this particular spot in the gene, and that’s shown at the top. The wild types are homozygous for the proper sequence. But then some of the cells in the brain are heterozygous for this mutation. And now you can see that yellow peak again, but now it’s much bigger. It’s about the same height as the normal peak. And so these cells have obtained, spontaneously, a new mutation, and it’s a dominant mutation because it causes trouble when you only have one copy of the mutation instead of two copies of the mutation. And it’s a dominant mutation that causes the cells to do something that they don’t ordinarily do. And so it pushes the cells, ... the stem cells to proliferate more, so the brain ends up getting bigger. And then it also pushes the neurons and the glia that are formed and that are not dividing anymore to function abnormally. They signal abnormally. The neurons don’t regulate their channels, their electrical channels, properly. And so this is what causes, then, the abnormal electrical activity and ultimately the seizures. And so this is a genetic mosaic mutation. This shows sort of schematically what development looks like in the fetal brain, and I’ll tell you more about this in the second half of the lecture.

The brain cells are formed from stem cells, which are those elongated looking cells at the bottom that are actually located in the inner surface of the brain. And they divide to form the post-mitotic neurons that form the adult brain. Then in Dante’s brain, what happens is this spontaneous mutation is present in some of the progenitor cells, and it causes them to divide too many times. So the brain ultimately ends up being too big. And so that ends the first part of the talk. I think I’ll stop now and ask if people have any questions about the material I’ve talked to you about first. Yeah.

[STUDENT:] So if it was just one hemisphere of the brain that became mutated, does that mean during development in the womb, that the two halves of the brain developed differently?

[DR. WALSH:] Well, the brain starts out as a single tube, actually, a hollow tube with fluid in the middle. And then the brain structure develops sort of as a lining of the tube. And then in the very front end of the brain that forms our cerebral cortex, that tube gets sort of branched almost in a Y fashion. And so the two hemispheres of the cerebral cortex get set aside relatively early on. And so that’s how mutations seem to be able to get mostly localized in one half of the brain and not the other. And of course, we don’t know that there’s not a single cell in that other hemisphere that has the mutation in it. Maybe there’s a couple on that side. We don’t formally know that. We just know that it’s not in his blood, but it is in his brain. Yeah.

[STUDENT:] Would there be any connection between hemimegalencephaly and an association with cancer?

[DR. WALSH:] So this is an example that I mentioned in the introduction where the AKT3 gene in other cell types, or actually other AKT genes—there’s a family of them—seem like they can become hyperactivated in cells that keep dividing. And it promotes them to keep dividing, and that can contribute to cancer. But in the brain, as I say, even though the gene is hyperactive, nonetheless, the
brain still has powerful mechanisms to force cells to stop dividing. And so the gene is still hyperactive even in the post-mitotic cells and seems to mess them up in different ways. And that’s where, without the ability to determine these genes, we wouldn’t have realized that a similar gene or the same gene is doing similar things but in totally different contexts. Yes, in the back there.

[STUDENT:] Would Dante have problems not only with fine motor skills, but with other things associated with the right side of the brain, such as creativity?

[DR. WALSH:] That’s a great question, and he certainly would have. And we don’t know, then, how much those activities can get taken over by his remaining left hemisphere. We know, for example, his left leg is ordinarily governed by the right hemisphere that’s removed, but he can walk. And so somehow, the remaining left hemisphere has learned to coordinate the left side of the body. And so some of his other activities are probably taken over by the left hemisphere as well. Hi, the woman there in the red shirt.

[STUDENT:] Knowing the genetic basis for hemimegalencephaly, what is the risk for it being inherited, like an inherited disorder?

[DR. WALSH:] So hemimegalencephaly is a genetic disorder, but it doesn’t seem to be inherited because it doesn’t, ... because you can only inherit things it they’re passed through the germ cells, if you have a mutation that gets into the sperm or gets into the eggs. And in his case, it looks like the mutation is present only in brain cells. And so the mutation actually occurred after the germ cells were set aside from the brain cells. So this never runs in families and seems not to be an inherited condition, although it is a genetic condition. And that’s another irony that we’ve learned, you know, relatively recently, that so many diseases that are not inherited still reflect the abnormal sequences of genes. Yeah.

[STUDENT:] Did you have any ethical concerns with removing one hemisphere of Dante’s brain?

[DR. WALSH:] Oh, well, obviously you can imagine that the idea of a radical surgery like this is something that families have to grapple with, and every family has their own feeling about it. And you know, even doctors are like, holy cow, you know. But the surgery has been around for probably 15 or 20 years and is being used more frequently rather than less frequently, because it just seems, as desperate as it is, it just seems like the best way out of a very tough situation. Yeah.

[STUDENT:] Has there been any like, artificial protein regulators that people are studying so that it can act like a competitive inhibition to prevent the hyperactive cells from actively engaging in mitosis?

[DR. WALSH:] Well, so that’s a great question. So this particular AKT3 is a gene against which, in the cancer field, medicines have been developed that actually damp down that pathway in the context of cancer. And so we’re actually optimistic that some of those drugs might help kids that have this condition. We don’t know if it’s going to spare them the surgery or not. But it’s something that at least we’re now looking at ways to try.
I wish I could take more questions, but I think I’ll have to move on at this point. I’ve told you about a developmental disorder where the brain fails to achieve its normal size and ends up too big because it gets too much of a push when the stem cells are dividing. And I just want to return to this slide that shows, that illustrates that these tremendous genetic tools have allowed many labs to understand a lot of these developmental disorders over the last several years. And in the second half of the talk, I’ll tell you about a different disorder, a complementary disorder. I’ll tell you a little bit more about disorders where the brain ends up too small, and that’s a condition known as microcephaly. And that’s illustrated by the small brains in the lower left corner and in the middle of the left where I put that box around it. I mentioned microcephaly briefly. It’s defined as a small head, actually. That’s why if any of you remember when you went to your pediatrician’s office, they put a tape measure right around your head as though they were measuring you for a hat. What they’re actually doing is they’re measuring your brain because our head is basically a carrying case for our brain. That’s the way we brain-centric neurologists like to think about it. And in fact, you can get a pretty good idea of how big a kid’s brain is by just putting a tape measure around the outside of their head.

And so this is a genetic condition. There are many different genes that cause it. And so that is what sometimes makes it a little difficult to find any one of the genes because there are so many different genes that can cause this condition. As you might imagine, with the brain being small, these children are lacking many of the neurons that they would normally have. And so they typically show intellectual disability. They don’t usually have seizures. Even though they don’t have enough neurons, the neurons that they have look like they work pretty well, so kids can typically learn to walk. They’ll have limited language. But they’ll be, as I said, somewhat limited in their cognitive capabilities. So most forms of microcephaly are not dominant mutations. They’re, in fact, recessive mutations, where you only get the disease if you disable both of the two copies of the gene that you carry, because we carry two copies of most of our genes that are on our autosomal chromosomes. And so these conditions are usually not spontaneous. They’re in fact usually inherited. And usually, the mutations occurred hundreds or thousands of years ago and are carried in a silent way in the population. And when two people who unknowingly carry the same recessive mutation have children together, they will have affected children.

And so this shows two families that had children with microcephaly: one from Mexico on the left and one from Turkey on the right. And I have a little dot inside of the square that represents the father, and I have a little dot inside the circle that represents the mother, indicating that we think that they were silent carriers of a recessive mutation. And then they were normal themselves but had the bad fortune to have three affected children. And this is, you know, a recessive condition; usually one out of four children are affected. But unfortunately, the statistics were very unkind to this particular family. But that’s what brought them to research.

And then another family from Turkey had a similar family structure. There are some normal siblings that are not illustrated for simplicity. And since the family from Mexico is from a rural area, we thought there was a good chance that they might actually share a distant common ancestor and that they might actually be silently carrying not only a recessive mutation, but the same recessive mutation in
the gene, and that the children would then be homozygous for two copies of the exact same mutation. And we thought the same might be true of the family from rural Turkey as well.

And so we set about trying to find this gene about 15 years ago. And the way we did that at that time was to do genetic mapping to localize the gene. The way you do that is by using markers to find your way. We basically had at that time a good map of the genome. And that map has gotten better, and so actually, I’ll illustrate some data from this family that was done in the early 2000s using markers, where we had 500,000 different markers across the genome. And by marker, I just mean that there are sequences of the genome that tend to vary between different people, so they are usually heterozygous.

And so what we’re looking for is a part of the genome where it looks like the kids inherited the exact same thing from both parents and are homozygous. And so this shows some of the markers on the left: the father’s chromosome has an A and the mother’s chromosome has a C, so that’s different. Way on the right, you see that the father has a G, the mom has an A. That’s different. But in the middle, the markers—even though usually people have different versions, there, the mom and the dad chromosomes are exactly the same. And so we found blocks of the genome that were homozygous that looked like they could have been inherited from that common ancestor. And this is actually what the actual output of that marker analysis shows. This is actually a representation of those markers across just one chromosome. This happens to be chromosome number 19. One end is on the left, the Q arm is on the right, and the centromere would be somewhere around the middle. And the markers show up as either red or blue when they’re homozygous and yellow when they’re heterozygous. And so what we’re looking for are parts of the genome that lack yellow color and that have large blocks of homozygosity. And we can use a statistical model that allows us to recognize those blocks of homozygosity more easily. And that’s shown here in the purple, where purple represents homozygous and yellow represents heterozygous.

And so what we do in families like this is we look for the homozygosity. And we start with all of the chromosomes, two copies of all of them—except, of course, the X and the Y in males. And we can take an individual person and do that single nucleotide marker array to find the blocks of homozygosity. And when we did that in the Mexican family, we found that the first child had a variety of blocks of homozygosity throughout the genome from various sizes—some big, some small. And that made us think yes, indeed, it looks like the parents do have a distant common ancestor and that our mutation is probably going to be in one of those homozygous blocks, but it doesn’t really eliminate that much of the genome. It gets us down maybe about to 10% of the genome. But then when we look for the overlapping blocks that are shared by two affected siblings, then that narrows it down to a much smaller segment of the genome. And in fact, when we compare the homozygous segments in all three of the affected kids, that got us down to just one block of homozygosity in the entire genome. And this is where we were, actually, in 1998. We knew that the gene was right there on chromosome 19. But you can see it’s actually a substantial portion of chromosome 19. So we know that our gene is going to be one of the genes in that interval, and it’ll have a homozygous mutation.
But now, I’ll show you a blowup of that region. And in fact, even that little homozygous region that looks so small actually has 6 million base pairs of DNA in it. And in fact, there were over 150 genes in there. And so in the early 2000s, we sort of had taken the project this far, and it was really not realistic and not something we could even afford to do to set about sequencing all 150 of those genes one-by-one with the old-fashioned technology. And so basically, we set this project aside on the shelf for a few years and let that graph of DNA sequencing costs take a few turns until the sequencing got cheaper. And we came back to it in the second half of the first decade of the 2000s because that’s when the next-generation sequencing made this project suddenly very easy, because around 2008, the project was picked up again, because now we had fast and inexpensive sequencing that could jump-start this research. We could then use these next-generation sequencing technologies and take all of the genes, all of the DNA in that segment of chromosome 19 and sequence them all, and then also analyze them in very high-throughput ways, because so many genomes had been sequenced at that time.

So from that segment, we found that there were over 2,000 genetic variants, places where the affected children’s DNA sequence was different from the consensus human sequences that were available on the Internet. And in fact, we could also find by looking at the genomic sequences of lots of normal people who were on the Internet at that time—Craig Venter’s DNA sequence, Jim Watson’s DNA sequence—were available on the Internet. We could say how many of these variants were not present in any of these normal people whose DNA sequence was on the Internet. And we found that there were only about 300 of these variants that were never seen in normal people, and we assumed that normal people would never carry these variants. And so that actually starts narrowing us down to relatively fewer possible mutations. And then we could start looking at, well, what do the different mutations do. Which of the mutations actually are likely to matter? Which might actually change something about a protein by causing a stop codon that might disrupt the size of the protein or by changing one amino acid to another that might change the function of the protein?

And so only seven of these changes that we had identified were potentially likely to be deleterious to encoded proteins. And so this just summarizes how we can go through this targeted sequencing, going from a large number of potential variants to a relatively small number of candidate genes that are shared by the three siblings of the first family from Mexico, but now we did exactly the same process in the second family from Turkey. So this allows us, then, a second opportunity to look at the intersection of the potential mutations in the two families. And so Family A had seven candidate mutations in this chromosome 19 interval. Family B had four potential mutations in that same interval. And only one gene was mutated in both families, and that’s a gene called WDR62. So by a very rapid process, we were able to take a lot of information and siphon it very quickly and funnel it down and find a single candidate gene.

And in fact, once we had that gene, we and other labs quickly found 20 other families that had other mutations in the same gene. In fact, this gene was discovered simultaneously by three different labs who were all, ... who had all been at the same place as us, stuck for five or more years looking for this gene because of the limitations of DNA sequencing, and then suddenly enabled. And so we were just waiting for the DNA sequencing to get better to find the gene.
So what does WDR62 actually do in the cell? We know that it regulates the size of the brain, and we know that regulating the size of the brain is really important. And so how does it actually function in the cells that make up the brain, because that’s really a lot of the biological interest of studying these disorders is also to tell us what it means about how the brain is formed. So we started analyzing where the protein is actually localized. And we found that it’s localized not in the neurons of the brain, but in the dividing cells of the brain. So it’s not a gene that functions in the neurons itself, but it’s a gene that functions in the stem cells, the dividing cells that give rise to those neurons. And WDR62 specifically localizes to the actual mitotic spindle of dividing cells. The left panel shows you what a mitotic cell looks like. And you’ve probably learned this in your biology textbooks. You can see that the actual spindle, the microtubules that make up the spindle, are outlined in red, because that’s an antibody that reacts with those spindle microtubules. You can see the blue shadows, which are the chromosomes, that are aligned along the middle of the spindle and just getting ready to be segregated. And then you can see the two ends of the spindle outlined in green, the centrosomes that organize the spindles. And we found that WDR62 is associated with these centrosomes of the mitotic spindle. In the right panel, now WDR62 is labeled in green. And you can see that that localized predominantly to the centrosomes at the outsides of the spindles. And in fact, WDR62 is not the first gene that had been identified to cause microcephaly. It was about the sixth or the seventh. And remarkably, many of these microcephaly proteins localize to the spindle.

Another microcephaly protein called ASPM, which I’ll tell you a bit more about in a few minutes, also localizes to the spindle, as do a half a dozen others. And so remarkably, it seems like there’s something very important about the spindle—and not the whole spindle, but just the centrosome, the organizing sites of the spindle—that seem to be very important for controlling brain size. And to tell you a bit more about how we think that works, I’ll tell you a little bit more about how the human brain develops by, if we can show this video that summarizes how the stem cells in the brain generate the cerebral cortex.

So this shows, then, a schematic movie through a fetus. And here is that developing cortex. The ventricle is the hollow, fluid-filled space. The cortex develops in the lining of that tube. And then if we look at a close-up, the fluid-filled space, the ventricle is down at the bottom, and that lining contains these neural stem cells that have these very elongated outer processes. They’re called radial neuroepithelial cells or radial glial cells. And they divide to form the post-mitotic cells, which are represented by those little bubbles that then look like they’re floating to the surface, because the post-mitotic, nondividing neurons actually migrate from the inner part of the brain to the outer part of the brain in what is actually a very complicated process all its own that we don’t have time to go into in detail.

So you can see the migrating cells going up. The first-born cells that first reach the cortex actually end up forming the bottom layer of the cortex. And the later-born neurons actually migrate past them. And so the last-born neurons of the cortex are always being added to the top, like layers of a cake. And so the very, very last-born neurons are in the very outermost part of the brain. The first-born neurons are now being shaded red. And those end up occupying the bottom of the brain. And so the longer these
stem cells divide, the greater the number of neurons that get added, and these last-born neurons tend to be added to the outermost part of the cortex.

And so looking again at these dividing cells, a variety of work from many labs suggests that exactly how those stem cells divide is very important in controlling how big the brain gets. When those cells are at an early stage of development, they tend to divide side-by-side with a horizontally oriented mitotic spindle to form two daughter cells that are also stem cells. So it’s stem cells generating stem cells, which grows the cell population exponentially. And that’s shown by that horizontal division on the left. A little bit later during development, the neurons start to be formed. And the neurons are post-mitotic. And so those cell divisions that generate the neurons or that generate one neuron tend to have a slightly different orientation. They divide to form one stem cell plus a post-mitotic cell. And so this actually results in a slower growth of the overall population because it’s linear growth rather than exponential growth. But eventually, in order to form neurons, you have to start having this linear phase. And these neuron-generating cell divisions tend to be oriented slightly differently and generate, as I said, post-mitotic cells. And so it looks like the orientation of that mitotic spindle is extremely important for the decisions that cells make about their fate. And the orientation of the spindle and other aspects of the centrosome that we don’t completely understand seem to control whether a cell takes on the fate of a dividing cell or whether a cell takes on the fate of a post-mitotic neuron.

And so experiments from a few different labs have shown that when you deplete a microcephaly gene called ASPM—and the same seems to be true for WDR62 as well, and other microcephaly genes—the mitotic spindle tends to turn a little bit. And it tends to turn to an angle where the cell divisions are more likely to generate post-mitotic cells. And this slight twist seems to have a very important consequence because by turning the mitotic spindle slightly, you form fewer stem cells and more post-mitotic cells. And so if you turn the spindle too early too often, you may form neurons at a stage earlier than you should and end up with a brain which is ultimately smaller than it should be. And so this mitotic spindle orientation seems to be very important to influence cell fate and cell proliferation.

So in the last few minutes, I’ll just tell you one unexpected aspect of this work that we think of as experiments in medical genetics and in developmental neuroscience. And it has to do with how the human brain evolved. The kids with microcephaly have a brain which is abnormally small, and people had long wondered whether some of the genes that, if they’re missing, make our brain end up being small might also have other sorts of ways in which they can differ from one species to another that might have helped make our brain big during earlier evolutionary time. So this figure here summarizes the brain size of humans way on the right, which is about 1,300 to 1,500 grams, compared to the brain size of our ancestors. And chimps have a brain size of about 400 grams. And then earlier human ancestors started at about 600 grams. And you can see how brain size progressively increased over the last several million years. And so this must represent some kind of genetic changes to something that allows the brain to get bigger but not suddenly, like Dante’s brain, and in an uncoordinated way like his brain, but in a gradual way that allows the new neurons to be properly integrated and properly functional.
And so it was long a hypothesis that genes that affect brain size might have also had this double evolutionary role. And so identifying some of the first microcephaly genes gave laboratories the chance to specifically address this hypothesis to see whether the microcephaly genes might play a role. And the way this sort of experiment is done is to take the same gene—ASPM or CEP63 or some of the other microcephaly genes that have these evolutionary roles—and sequence them in humans who have a brain size of about 1,300 or 1,400 grams, and then compare it to the DNA sequence in our closest existing relatives and see how similar they are. If the gene sequence is exactly the same in all of the different species, then you wouldn’t expect that it has any particular role in evolution. But a lot of genes are actually very highly conserved. But maybe the gene has more differences than you might expect, and that might suggest the gene actually plays a role in making those species different.

And so in fact, when that sort of analysis was done for ASPM and for one or two other microcephaly genes as well, there was an excess of differences in the gene, particularly in the lineage of beings that lead to humans, suggesting, and strongly suggesting, that these genes might have played an evolutionary role. And so this is remarkable because it gives us a way of thinking about how the evolution of our brain happened, which ... you know, of actually thinking about how those genes acting over millennia might have actually worked in the cell biological way to make our brain bigger than that of other species. And it may come down to something like this control of cell fate and proliferation that something as simple as a little tweak in the angle of a mitotic spindle might allow a brain to become larger, but in a very organized way. And so if there’s a little bit of a turn of the mitotic spindle due to perhaps some subtle functional changes in microcephaly genes, that might allow these stem cells to divide just a couple more times in the human brain compared to the brain of other species. But what happens with those couple of extra cell divisions is that you’re adding a few new neurons to the top of the cortex, but you’re adding neurons that don’t exist anywhere else in nature. You’re adding something completely new. And then those new neurons have the ability to potentially take on functions that are not served by cortical neurons in other species.

So just to conclude, then, these structural abnormalities are an important cause of human disability. You don’t hear about them quite as often as autism or other ... or intellectual disability because they’re individually quite rare and because the kids are often severely disabled. But we can learn a lot about them by understanding their genetic causes. And then finally, surprisingly, some of the genes that cause structural brain abnormalities were targets of brain evolution. They control the shape of our brains, and they allow our brain to be shaped differently and to do amazing, different things that the brain of other species can’t do. So why don’t I stop here and take questions on both parts of the talk? Yes, in the back.

[STUDENT:] How does the skull know to stop growing based on how big the brain is?

[DR. WALSH:] That’s a great question, and that’s a complete mystery. When the brain is first growing, the actual skull forms as plates. And the plates are connected by just soft tissue, and so they sort of float on top of the brain. And the plates start fusing together around the time of birth, and they don’t complete fusing until after birth. And so actually, when the baby is born, those plates actually scrunch a little bit and can even overlap to allow the baby to exit the birth canal. And we don’t know exactly
what matches the bones to the brain so perfectly. But obviously, it’s a problem. There are occasional conditions where that soft tissue starts turning to bone too soon, and the brain starts getting squeezed, and that can be a big problem, as you can imagine. Yeah.

[STUDENT:] So if megalencephaly can, like, develop during the developmental stages of pregnancy rather than be inherited, is the same true for microcephaly?

[DR. WALSH:] It can; ... most of the causes of microcephaly look like they’re recessive, but there’s that one cause that’s associated with that deletion of the end of chromosome 1. And that—as far as we can tell, that’s always been present as a mutation in the germ line, meaning that it happened in a sperm or an egg. But I suppose it’s possible that it could happen as a somatic mutation, but we wouldn’t have detected it, because those kids don’t get surgery. So we can’t study their brain tissue, so we don’t really know.

[STUDENT:] I know this is a very controversial subject, but based on your research and on the evolutionary theory, do you believe that our genome, the size of our brain, or the structure of our neurons might play a role in intelligence?

[DR. WALSH:] Well, I don’t think that’s controversial. I don’t think anyone would disagree that the structure of our brain is important for determining intelligence. A lot of people ask, you know, are people with big brains smarter than people with small brains, you know, and Lord Byron had a brain of 2,000 grams, and Walt Whitman had a brain of 1,000 grams, and so people can argue who was the greater poet. We think that there’s a general relationship between brain size and intelligence, and studies have shown that. But it’s by no means absolute. And some people who have brains that are one or two standard deviations below normal can be absolutely as highly intelligent as anyone else. Thanks very much for your attention.

[applause]

[music plays]