[music plays]

[ANNOUNCER:] 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 third lecture is titled "Decoding the autism Puzzle." And now a brief video to introduce our lecturer, Dr. Christopher Walsh.

[music plays]

[DR. SAWYERS:] This tremendous capability of high-throughput genome sequencing has all kinds of applications for understanding how the human brain develops and how abnormal development can cause disease. At the moment, we are really excited about sequencing, not just the genome of a person, but the genome of a single cell and comparing how the genome of one cell differs from the genome of another cell. We are increasingly starting to think that every cell in our body has a bar code that consists of the unique mutations—most of them silent—that occur as that cell develops. What that means is that our whole body carries a history. There is a permanent record in the genome of the cells in our body of exactly how our body developed. It's a family tree. The cells in our body have in their genomes a family tree that, in principle, would allow us to reconstruct the entire pattern of cell divisions that generates each one of us. What this will allow us to do is understand exactly how the human brain developed over time. Someday we will be able to compare the pattern of development of a normal brain with the development of an abnormal brain. Autism is an example of a condition where the brain appears to develop normally but seems to function very poorly in terms of that person being able to learn normal socialization. The problem that kids with autism have looks like it's not in the cells themselves, but in how those cells communicate. It's as though you had all the houses in the neighborhood set up in the right place, but there was no phone lines and those connections are so tiny that they can't be captured without a microscope.

On the other hand, this absence of gross defects in shape and form make us hopeful that we will be able to do a better job of treating autism because it looks like all the players might be there and we just have to start getting them to work better together.

[applause]

[DR. WALSH:] Well, good morning everybody, welcome back. It's great to be here and I'm really excited about giving you this second lecture on the general topic of developmental brain disorders, and this time talking about how much we've learned about autism and autism spectrum disorders in the last ten years or so. And I hope to show you over the course of this lecture how we've really gone from, just ten years ago, understanding very little about the causes of autism and being really mired in a lot of controversies about what many of those causes might be, to coming to a growing consensus about the kinds of mechanisms that cause autism, a growing understanding about how those mechanisms play out in the brain and a growing momentum to try to use our understanding of those biological mechanisms to develop better treatments and that's something that I hope maybe some of you in your

future careers in science might be able to contribute to. So as I mentioned already in the introductory video, autism is one of a variety of disorders that don't show abnormalities when you put a patient in an MRI scanner. Generally, most kids with autism spectrum disorders will have a brain that might look much like yours and mine and that's common. There are plenty of other childhood brain disorders that also show a normal-appearing outward brain, but a brain that doesn't work the way it should be. Can any of you think of any other conditions that might affect children or young adults where you might have heard that the brain doesn't show a structural abnormality but it still has functional problems? One … defects in communication or thought. Yeah?

[STUDENT:] Depression.

[DR. WALSH:] Yeah, that's a great example, where again as far as we can tell, the brain doesn't show any outward structural defects at all. Yeah?

[STUDENT:] Dyslexia and other learning disabilities along that area.

[DR. WALSH:] Excellent. All right, anyone else have any other thoughts? Yeah?

[STUDENT:] Obsessive-compulsive disorder.

[DR. WALSH:] Yes, another great example. So there are a variety of these conditions where the brain didn't develop properly but it doesn't develop in an improper way that's obvious anatomically, and so I'll be focusing during this lecture specifically on autism and autism spectrum disorders. And so first I just want to define, what is autism. So autism is a behavioral condition. It's something that you diagnose by observing a child, and it's diagnosed by a triad of abnormalities of social interaction. First there are impaired social interactions, things like poor eye contact or a general disinterest in people. Autistic kids typically like to do things rather than interact with people. Sometimes they are extremely fearful of contact. And this is, in many ways, one of the saddest things about autism, because often parents find that they can't hug their kids who are on the autism spectrum because the children can be very sensitive to that. They also show defects in communication, especially language, so typically, autistic kids may be initially diagnosed because they fail to obtain language at the usual age, which is typically about two years of age. And the third diagnostic criteria is the presence of repetitive or stereotype behaviors. Autistic kids will frequently have unusual interests or they may have repetitive movements, things like shaking or head banging. And so it's really by watching kids that the diagnosis is made. There's not a blood test, there's not an MRI test like there is for cancer or structural brain abnormalities.

And so in many ways also, this makes the diagnosis of autism somewhat imperfect. The term "autism" is sometimes used a little bit differently by different people. And also we find that some kids who are initially given a diagnosis of autism may grow out of it because their social interactions improve later in life. So autism is really part of a spectrum of disorders. And so pure autism itself is just one of a number of related conditions we refer to as autism spectrum disorders. Collectively, autism spectrum disorders affect as many as one out of 88 children. But they range tremendously in the kinds of effects that they have on kids and young adults. They can range from the very milder forms of autism that are

commonly referred to as Asperger's syndrome, where children can be highly intelligent but socially very awkward. The sort of thing you might have seen on "The Big Bang Theory," Sheldon, would be an example of someone who would be a very intelligent person but who has very severely impaired social interactions. All the way to kids who have problems in all sorts of spheres, not only social spheres but have severe problems with intellectual disability as well and may be completely unable to live independently and may require extensive home care or institutionalization.

And so now, the term autism is used to refer to all of these various conditions, and that makes it sometimes scientifically difficult to study. We understand that there is a tremendous amount of variability hidden under that single term, but in the case of those structural brain abnormalities we can see the variability because we can see the different ways in which the structure is abnormal. Here, we know there is an underlying variability, but it's hard to know exactly how to categorize various children that are on the autism spectrum.

So what are the various things that might cause autism? You may have heard of different things that people have suggested that might increase the risk of autism in the press or anywhere else, and anybody want to volunteer any things that you might have heard about? Yeah.

[STUDENT:] Like when you're pregnant and you eat a lot of mercury?

[DR. WALSH:] Yes, that's poss- the possibility that there might be some sort of environmental toxin as a cause of autism. In the back.

[STUDENT:] When the umbilical cord gets tangled around the neck of a child or baby's …

[DR. WALSH:] Yeah, so some sort of brain damage to the fetus due to lack of oxygen. Yeah.

[STUDENT:] Like disruptions in protein productions for like vasopressin or oxytocin.

[DR. WALSH:] Uh-huh. Right. Yes?

[STUDENT:] Some lead-based paints in young children sometimes can cause autism.

[DR. WALSH:] So other sorts of toxins. Yeah. You?

[STUDENT:] There's age of the father.

[DR. WALSH:] Yes, that's a very interesting and a very good suggestion. Yeah?

[STUDENT:] Vaccines.

[DR. WALSH:] Right. So vaccines are another thing that has been suggested to cause autism and that has been … that particularly was an area that has been very controversial over the last decade or so but there is a growing consensus that vaccines don't seem to significantly increase the risk of autism despite the persistent belief among some people that they may do. And I'll show you an example of one study that specifically addressed the possibility that vaccines might cause autism. They studied large numbers of kids, either that were vaccinated or not vaccinated. And they studied the rates of

autism spectrum disorder, which is abbreviated A.S.D. down there at the bottom, and you see that in the kids that were not vaccinated, in fact, the rates of autism were nominally slightly higher but there is no significant difference. And in fact, because of the many concerns about the relationship of vaccination to autism, there have been many large studies like this one that have shown no significant link between vaccines and autism. And in fact, some of the early small studies that claimed an important link, some of them have even been retracted because it has been found that, in fact, the science was not very good behind that studies.

So how would we go about determining whether genes might play a role in autism? In fact, we've known for more than a decade that there is a large genetic component to autism. Long before we knew what those genes might be or how they might act, there was already strong evidence that there was a major role for genes in the causation of autism, and that evidence came from studies of identical twins, because identical twins share the same genome but they can be potentially exposed to different environmental factors.

And so, just to review that, a variety of twin studies have compared the concordance of autism spectrum disorders in twins that are either nonidentical or identical. And concordance just means if one twin is affected, what's the risk that the other twin will be similarly affected with a disorder on the autism spectrum. As you know, nonidentical twins share about 50% of their genes. They're exposed to similar environments as one another, both before birth and after birth, and they actually show an increased concordance for autism which is much higher than the general population. 'Cause I told you that the risk of autism in the general population is about 1%, but fraternal twins have a rate that's ten or 20 times that, so in fact there must be some inherited factors that act even in fraternal twins, and there's an increased risk in siblings as well. So that implicates some genetic factors but identical twins—you can see that concordance rate is extremely high, anywhere from 70%-90%. Again, very strongly implicating genetic factors as causative of autism spectrum disorders. And these data have been replicated many times and as I say, they were available before we even started having the technologies to understand what those genetic causes might be.

So what sorts of genetic mechanisms might cause autism? And you've been hearing about various forms of genetic inheritance from my talk yesterday and from Dr. Sawyers' talk yesterday. Genetic disorders can be recessive, dominant or can be more complex and multigenic in their causation. So we heard yesterday that single gene recessive disorders, where you only will get a disease if you inactivate both of the copies of a gene—the one you inherit from your mother and the one you inherit from your father. You also heard about single-gene dominant disorders, where a mutation of just one of the two copies of a gene is sufficient to cause a disease, either because the mutation overly activates the gene or because that gene is sensitive to the dosage—because it requires two copies for its normal activity. And some examples are given here. And we haven't really touched much on these multigenic or complex traits where there might be multiple contributing genes, but in fact, the increase in risk from any one of those variants is relatively small, and so those common variants are carried in the normal population.

And we know that there are many diseases, particularly diseases of adult life, that have a large role of these common variants that seem to interact with one another. And it's formally possible that the same might be important in autism spectrum disorders as well.

Now before going into the specifics of the genetic studies of autism spectrum disorders, I just want to remind you that not all genetic traits are inherited, and I think that's something that's come out very strongly in all the lectures so far. This shows two people, a mother and a father, the father in blue and the mother's chromosomes in pink, and they seem to be normal without a mutation. They have four children and again, roughly half of the child's genetic material comes from the father and the other half comes from the mother, but sometimes you can have actually an error in the copying of DNA. In this case, it would be in the formation of one of the mother's eggs, and this can create a de novo mutation. So it's detectable in the child, but it's not detectable in the parent.

And these, sort of, dominant mutations, but that are not inherited, but that are spontaneous and de novo are a surprisingly common cause of disorders, particularly of children, since they particularly, of disorders that impair the ability of that child to have a family themselves. So as recently as about 2005 or 2006, we knew very little about the causes of autism. People would say that it had a strong genetic cause, but then people would counter, well if it's so strongly genetic, what are the genes that cause it? And at that time we really only knew of a handful of rare single gene disorders, conditions like fragile X syndrome or tuberous sclerosis, and a couple of severe chromosome rearrangements that were associated with autism spectrum disorders. And these single-gene disorders were expensive to test for in an individual child, and they very rarely gave an answer, even fewer than in about 1% of the children, so in fact the existing genetic tests weren't widely utilized or frequently not paid for by insurance, and so … and physicians really didn't have a sense it was that important to test them, and so kids with autism were not routinely tested for even the existing known genetic associations.

And so the story of our growing understanding of the genetics of autism, it really is one about technological innovation again. Where the availability of better technologies has allowed us to do things that we couldn't do before, and as the technology progressively improved, so has our understanding of the condition. And so, one of the first technical innovations occurred right around 2005 and was a direct consequence of having a complete human genome sequence a few years earlier, and what having a complete genome sequence allowed was it gave us an ability to probe the entire genome to determine just whether there were small pieces of the genome that were missing or duplicated. Because deletions or duplications of the genome are an important cause of disease, they are collectively referred to as copy number variants, and they're illustrated here by this example. You can have in one case, a loss or a deletion of a segment of a chromosome; in other cases, you can have a duplication of a part of a chromosome. This can potentially lead to overexpression of a gene product, and we saw that yesterday in some of the children that have hemimegalencephaly, where they have a duplication, a very large duplication of half of a chromosome.

Now copy number variants are not invariably disease-causing. For example, you might have a small deletion that only deletes some of your junk DNA and doesn't necessarily actually affect a gene because most of our genome actually doesn't code for proteins. Or you might delete one copy of a

gene, but that gene would cause a recessive disease, so a loss of one copy would not necessarily be a problem. Or similarly, some genes are not sensitive to being duplicated. On the other hand, large copy number variants will frequently delete many genes and if one of those genes is dosage-sensitive, then that can immediately create a problem.

And so with the availability of genome sequence in the early part of last decade, this allowed the development of assays where you would actually immobilize segments of the entire genome on a glass slide and then hybridize that to a patient's DNA and compare it to a normal reference person and then you can measure very, very precisely whether small parts of the genome are missing or duplicated. You can see that you will get a green signal, which would mean a part of a genome is missing where the reference DNA is greater than the patient's DNA; a yellow signal, means that the red and the green are equal and the patient is normal; or a red signal, which would imply that there is more of that segment from the patient than from the reference, meaning that there is an amplification or duplication.

So with the availability of these technologies, these are applied to large numbers of children on the autism spectrum and the results of one such study is summarized here. So in this study, what we're doing is we're comparing an autistic son, who is represented by the shaded square, to his normal sibling, in this case a sister. And what we're looking for are copy number variants that are present in the child but not present in the parent. And so we refer to them as de novo copy number variants, and we are particularly interested in ones that are rare because we know that, since not all copy number variants are disease-causing—in fact, all of us have copy number variants that distinguish some of us from the other that are just silent ones.

And so this study focused on rare ones, particularly those that deleted or duplicated genes. And you can see that the effect is quite striking, that in the affected children, they had … between 5% and 6% of them had a rare de novo duplication or deletion, somewhat more commonly deletions than duplications, and the number was far in excess of what you would see in their unaffected siblings.

On the other hand, unaffected siblings do occasionally have these copy number variants, so not every single one of these rare de novo copy number variants is necessarily causative, but the majority of them are.

Now this actually was a real turning point, because now we find that from this study and other studies like it, we find that about 5% or 6% of kids with autism can have a single diagnostic test, … this copy number variant analysis, which is sometimes referred to as a chromosome microarray, essentially doubling the number of kids that can be diagnosed. And this study was really a turning point for several reasons. It inspired, actually, a large group of us in Boston funded by local philanthropists called the Autism Consortium, to do a very large study in the clinic of changing the way we used genetic tests. Instead of first diagnosing a kid psychologically on the autism spectrum and only secondarily thinking about whether it might be genetic or not, we decided, let's do genetics first, and every kid that walks into the clinic on the autism spectrum, let's immediately test them for copy number variants with this test.

And in fact, in our own study from all of the pediatric institutions in Boston and from almost 1000 kids, we found that about 7% of our kids had a diagnosable copy number variant that was causative of their condition. And studies like that actually led the American College of Medical Genetics and the American Academy of Pediatrics to both make formal recommendations that copy number variant testing should be part of the initial evaluation of any child on the autism spectrum. Also, these studies led to the description of new specific genetic syndromes: particular parts of the genome that were recurrently abnormal to cause autism spectrum disorders—chromosome 16, chromosome 15, chromosome 1.

And finally it also caused a cultural change that I could see among my colleagues. Many developmental psychologists or pediatricians who had not really absorbed in their head that it was a genetic disease, had been following kids for five years or more and suddenly found that that kid had a deletion of 16p, p11 and we're suddenly acting like autism was a genetic disease, not just talking like it was a genetic disease. Now these are all typically de novo mutations, and if de novo mutations are important, is there a role for, not just these large gross rearrangements of the genome, but finer, smaller things like point mutations. And that was again where the technology really enabled us to take the next step. Because with the availability of affordable whole exome sequencing, it allowed the first studies that really probed whether de novo point mutations might be important as well.

And so whole exome sequencing, just to remind you, sequences not the whole genome, which is five or ten times more expensive, but just focuses on the coding part of the genome because that is where most of the disease-causing mutations lie. And it was really then only as you saw yesterday, between about 2005 and 2010, that this was an experiment that people could even think about, to sequence the entire exome of patients and their parents to examine these de novo point mutations.

So point mutations are changes to the genetic sequence that can affect protein structure, and you can have all different kinds of point mutations, starting with a normal DNA sequence represented here in the DNA code TAC, although you know that codons actually are represented by RNA which uses uracil, but they're frequently recorded as their DNA product. A TAC codon would represent tyrosine, and so it can be mutated in various ways; if you change the C to a T, you can change the tyrosine at the DNA level but not at the protein level. If you change the middle A to a G, you're creating a missense change which is a cysteine, or if you change that final C to a G that creates a stop codon which would truncate the protein altogether.

And so this shows the genetic code as sort of a roulette wheel, and so what happens is as you create mutations, the most common sorts of mutations will swap one amino acid from another, because you can see that most of the codons code for amino acids, and so if you change something, the chances are you are going to change one amino acid to another. Less commonly, like this, you'll have a silent change. Most commonly, you'll get a missense change, and then very rarely, you can get one of these nonsense changes so that, because as you can see, there are only three stops on that wheel that represents stop codons, and so the least likely sort of mutation you'll get is a stop codon, but of course the stop codons, although they're rare, they are the most deleterious, because they almost invariably inactivate the protein.

And so this summarizes the results of a study that was designed similarly to the copy number variants study but instead used whole exome sequencing to look for point mutations. And again, they compared de novo point mutations in children who are affected compared to their normal siblings. And what you can see on the right side is that the overall rates of mutation in affected patients and their siblings is about the same. They both had it, on the average, about one de novo mutation in their exome per generation, and this has been found by many different studies. So if you add up the red bars and if you add up the blue bars, the overall rates of mutation are about the same. Meaning that autistic kids don't have a problem with DNA replication, by and large, the DNA polymerase works okay. But what you may notice is that the distribution of those mutations are different. So in fact, the autism kids don't have a higher rate of mutations, but what they seem to be is just unlucky in where those mutations fall on the roulette wheel.

So that the unaffected siblings who are represented by blue, they had a slight excess of silent mutations. The affected children had a slight excess of missense mutations, but the affected children also had a very large excess of those rare nonsense mutations. And nonsense mutations are rare overall, but you see that there is the greatest differential between affected children and unaffected siblings. The difference here is about three-fold, and with a large enough sample of children affected, this difference is highly statistically significant, suggesting that in fact, these de novo mutations as a group are another very important cause of autism spectrum disorders.

And I think I'll stop at this point and take questions. Yeah, in the back.

[STUDENT:] The DSM-5 was supposed to publish certain autism … it retracted certain diagnoses of autism. What's your opinion on that?

[DR. WALSH:] Well, I'm not part of the board that's making those recommendations. I think what they're trying to do is they're trying to achieve greater precision and they're trying to, as much as possible, decrease that overlap, I think, between autism spectrum disorders and the normal. And I think trying to achieve greater precision is always a good idea. Behind you, the woman right behind you. Yeah.

[STUDENT:] I was wondering if autism … did you feel, because of the genome effects and because of the possible environmental effects, is part of the nature versus nurture debate?

[DR. WALSH:] That's a fascinating question, and you've really put your head on it. I think the key, because it is a disorder in communication, so it's a disorder that affects how the brain interacts with its nurture and ye… and it has genetic causes, but it definitely has environmental influences, because we know that if you take a kid on the autism spectrum and give them intense teaching and intense training, you can make them better. And so we know that autism can respond and can improve but with environmental factors as well. Yeah.

[STUDENT:] Do you believe or is there evidence that shows that severely autistic kids have heightened ability with things such as numbers?

[DR. WALSH:] Well, there are definitely autistic savants. There are definitely kids who have very, very poor social interaction and can be brilliant with numbers. We don't know how … that is, you know, movies like Rain Man and so forth have developed those people as characters—that definitely occurs and that's one of the biggest mysteries in neuroscience is how that can happen. How people can be so good in some areas and so poor in other areas. We don't know, … but most kids with autism spectrum disorders are not quite like that. They tend to have, most of them have more impairment that that. Behind, yes, right behind.

[STUDENT:] Can a de novo mutation only take place on the chromosome inherited from the mother, and if so, why?

[DR. WALSH:] Oh, no. In fact, most de novo mutations come from the father. They are several times more common in sperm than eggs, because the sperm are generated throughout life and most of the eggs are laid down relatively early, and so in fact, in the front row in our last question session, someone had suggested that paternal age might be an important cause and in fact, older paternal age is associated with a higher risk of autism because the sperm seem to accumulate more de novo mutations with age. Yeah.

[STUDENT:] Are copy number variant tests used to diagnose autism, and if not, why are they not used as a part of …

[DR. WALSH:] So now, copy number variant tests are … really are becoming a routine part of practice. They certainly are at our institution because we were heavily influenced by our own findings in that study. They are not always paid for by insurance, unfortunately. In fact, our group, the Autism Consortium, has been spending time at the state house in Massachusetts to try to make sure that it's always paid for because we think it's very important. There also have been studies that have shown that these copy number tests can influence medical management, and that's really the most important thing to demonstrate about such a test. Yeah.

[STUDENT:] I was wondering, kind of a follow-up on that question, why it's important for these … practically speaking, for a physician diagnosing a patient with autism, if there is such a large spectrum, why it's important to pinpoint any specific cause of the disease and if that can really help how a physician would treat or provide help to a patient.

[DR. WALSH:] So, that's a very good question. What is the point of all this? It's a research project to understand the disease, and we're trying to get at the mechanisms because understanding genetic mechanisms as we see so beautifully illustrated in cancer is a way to develop better treatments. And so that's really what we're after.

[STUDENT:] So perhaps working towards a cure, hopefully …

[DR. WALSH:] Exactly. Yeah. I'm afraid I'll have to go on and continue with the second half of the lecture at this point, but I wanted to start the second half by coming back to this slide and reminding you that, although there is a great excess of these severe mutations in affected children compared to

their unaffected siblings, everybody has mutations. The unaffected siblings also have even some of these very severe-looking nonsense mutations, and even de novo ones occur in the unaffected siblings. So unlike the copy number variant test, which is already really ready to be used as a clinical test because it's actually pretty accurate and the labs can do a good job of interpreting what copy number variants are likely to be causative and which are likely to be just a normal population variants, we're not yet at that stage with understanding how to apply whole exome sequences to the diagnosis of kids because we see that a lot of unaffected siblings have these apparent, these obvious, de novo mutations as well, that … and many of them look just as scary as the mutations that you see in the affected kids.

And so to understand then, which of these mutations are relevant, we have to do more work. And an example of a very recent study which is trying to further validate some of these candidate autism genes is illustrated here, where now not 800 autistic children, but over 2,000 autistic children were sequenced for a list of genes, and the names are given at the bottom, that were identified as having these rare de novo point mutations in children from the first studies and they said, okay, well let's test these genes and see which ones really validate in a larger population by being mutated in more than one child. And then we can have a greater sense of confidence that that gene is really on the list of genes that can cause autism spectrum disorders. And so what you see is that of all of the candidate genes that they resequenced, some of them, like CHD8, have very, very high levels of statistical likelihood of being an autism gene. Others a little lower, and a little lower, and then some of the ones, in fact that they resequenced, don't allow us to say for sure that say that LAMC3 at the right end is really an autism gene or whether that was some sort of accidental association.

So we are still a long way, I think, or at least several years from being able to reliably have a panel of autism genes and to be able to interpret this for the individual child who comes into the clinic, whether they have a particular point mutation, which is causative or not. And so, really then, this pie chart of the possible causes of autism has expanded greatly in just less than ten years. We've gone from understanding really just a couple of percent of kids to now expanding to these copy number variants, which as I say, are something which is really widely utilized clinically, and then an understanding that de novo point mutations as a group are probably even more important as causes of autism, although we're not yet ready, I think, to widely apply this in the clinic. But you can see we still have a long way to go, to understand even the 50% or 60% or 90% of the disorder that we think has a heritable cause. And on the other hand, the de novo point mutations, they don't really fully explain the heritability of autism. It's likely that many identical twins share the same de novo mutations since they derived from a single fertilized egg, so the mutations that come in the sperm or the egg are probably present in both of the identical twins, but de novo mutations certainly would not explain the increased risk of fraternal twins, or the increased risk of siblings. And so we know if there's large heritable components that still haven't been identified and the … actually, the study of these heritable components is actually more difficult, because we know that the disease has so many possible genetic causes, it makes it very difficult to pool information from different families to try to identify genetic causes by pooling different families because different families probably have different genes at work. And so one way to try to

identify some of these rare heritable causes is to find the unusual families that have multiple affected children and particularly families like this one that actually share common ancestry.

So here you see a father and a mother who are related to one another as cousins and have three children on the autism spectrum, and of course these sorts of families are rare, but in fact, when you can find them, they are extremely informative, because they sometimes allow you to go from a single family to identifying a single causative gene, and I'll illustrate an example. It also allows me to show you how the hunt for genes has been changed with the widespread utility of whole exome sequence.

Yesterday I told you that in the hunt for that microcephaly gene, we went through an exhaustive series of mapping and we mapped the gene to chromosome 19 and had to wait ten years before we had ways of going further. In this case, we can just take the DNA from the patients, the affected siblings and some of the normal siblings, and just go directly to whole exome sequence. And instead of trying to map the gene, we can just identify all of the variants in the entire exome and then analyze them as a single pipeline. And so in this case whole exome sequencing from three affected children shows that each of those children have lots of variants—about 25,000—compared to the reference genome.

But in fact they share relatively few, they share about 8,000 variants and of those, 53 of them are potentially deleterious, in that they might affect a protein, either by creating a missense or a nonsense mutation. But in fact, when you then trace down all of those potentially deleterious ones in this particular family, only one possibly deleterious mutation was homozygous in all of the affected children, and was not homozygous in any of the unaffected family members, and that was a gene called SYNE1. And so that study and other studies have suggested that recessive mutations are an additional piece of this pie chart, and they probably account for at least 5% of autism spectrum disorders, and that's not only true of these rare, unusual families but that seems to be true in American families with autism spectrum disorders as well.

And so now we have a growing list of autism genes, and that has in turn allowed us to now really start to think about what is the underlying cellular mechanism. Why does the autism brain show a brain that a has a normal shape and size but just doesn't work well, and how can we utilize our understanding of mechanisms to try to develop better treatments?

And that's illustrated in this video, so if we can run the video, I can walk you through how we think many of these autism genes work, and they have to do with the communication of one neuron with another. So there's a neuron here shown in blue, the myelin of the axon is shown in yellow, and then neurons communicate to one another. They carry electrical signals and then the electrical signals from one cell is transmitted to the other by a chemical signal at a synapse. And these synapses are the critical points of communication of one cell with another, and they actually change in response to learning. And this shows a close up of those synapses.

And so, for example, there are mutations in genes that connect neurons to one another through the synapse or mutations in genes that encode the proteins inside the spines, as they are called, that regulate the receiving of those signals. When you look at the proteins of the synapses, they are littered with proteins in which mutations have been found to be associated with autism spectrum disorders. So

neurexin, neuroligin are encoded by genes that are subject to autism mutations, Shank3 is one of the most commonly mutated genes in autism spectrum disorders; GRIN2B also as well. And we know also that when these synapses are not properly managed, that they tend to shrink, so that the electrical signals from one cell are not properly conducted by the chemical signals through the synapse to the second cell. And also we … there are other autism mutations, actually, that seem to act in the nucleus of neurons, but their action in the nucleus seems to be to regulate the expression of these genes and hence proteins that also regulate the health and the maintenance of the synapses.

Just to show you an example again, returning to that SYNE1. SYNE1 encodes a particular form called CPG2 that seems to regulate the structure of synapses and that was known actually before the gene had been associated with autism spectrum disorders. And so in the bottom panels, you see a close-up of some of these spines on the neurons that achieve these beautiful shapes, and it's the regulation of the shape of those spines that seems to allow a neuron to tune its synapses, to tune its connections in or tune certain connections out, by modulating the shape, the length, and the width of those spines, and you can see that CPG2 is located right in those spines, and in the absence of SYNE1, in fact, or CPG2, the shape of those spines changes. Here is an example of, in fact, how those synaptic shapes tend to change. This is from a paper that described the results of the absence of another autism gene called Shank3, and what they found was that, on average, these synaptic spines that receive the synapses from their partner cell tend to shrink and they're also less active electrically.

So this then comes back to summarizing where we've come in just really the last half a dozen years or so, to an immense increase in our understanding of the genetic mechanisms. An appreciation of the important role of these de novo rare mutations that can have large effects; an appreciation of the role of certain inherited mutations, the recessive ones; and again, like I said, a real cultural change, to understand that this is a genetic disease in a large fraction of the kids, perhaps the majority, and that we should start treating it like a genetic disease. We should offer genetic testing, at least the copy number variant analysis, and we should consider genetic counseling more often than we do.

Now, one of the puzzling things about the genes that have been associated with autism so far is that none of them seem to cause only autism. I think, when we started this out, there was a notion that we would find mutations that would do nothing but cause autism, that would be specific to regulating social behavior, but that wouldn't do anything else. But in fact, mutations that cause autism, whether they're copy number variants or point mutations, can cause other diseases as well. One patient might carry a particular mutation and show prominent autism. Another patient might carry seemingly the exact same mutation and the exact same copy number variant and instead might present with intellectual disability but retained social function, or schizophrenia, or obesity, or attention deficit disorder.

And so, the genetics hasn't yet explained to us the particular social defects that autistic kids suffer from. It's a puzzle why common genes can cause a diversity of functions except that all of these different diseases impair, in some ways, the function of the brain. And so it may be that the genetic mutations set up the brain for challenges, and that those challenges can be manifested in a variety of different ways.

And so then, where are we going to go in the next five or ten years? Where will the rest of the genetic pie chart or the mechanistic pie chart behind autism spectrum disorders end up? And of course, no one has a crystal ball; we can only speculate about what we're likely to find. The progress is extremely fast. Many of the studies I just described to you were only published within the last year or two, and so it's a very dynamic time and a very, very optimistic time for understanding a very difficult disease. So there are a variety of possible mechanisms that might be important: Common variants, other recessive mutations, somatic mutations, may help us build to perhaps more than half of the puzzle, maybe even as high as the identical twin studies would suggest, of two-thirds or more. Common variants are probably very important. In common variants, no one gene determines the phenotype but instead two or more genes might show various interactions to create a phenotype. These sorts of studies require large numbers of patients in order to find effects and so early studies of common variants and autism spectrum disorders utilized relatively small numbers of patients and didn't find clear effects. As the numbers are getting higher, some of those roles of common variants seem to be coming out. In larger studies of schizophrenia for example, which has many similarities to autism, have shown effects of these common variants.

As I was mentioning in the video before this talk, I've been fascinated with the possibility that, since meeting these kids with hemimegalencephaly, where you have a mutation that's in the brain, but not necessarily detectable in the blood, could there be other diseases that are caused by mutations that are in the brain, but not necessarily detectable in the blood. Again, with hemimegalencephaly, you have a mutation that is only in the brain but it immediately declares itself. It makes the brain really big, and it creates intractable epilepsy, and so it's relatively easy to understand that mutation in the brain, but mutations in the brain that cause defects with synapses would not create a structural problem, they would only create a functional problem.

And so they're, at the moment, really very difficult to study; we have very limited availability of postmortem autism brains, and so there might be these complicated developmental genetic mechanisms that may take many years to understand. But the likelihood is, if we discover those mechanisms, they're probably going to involve the same genes that we've already been able to identify by studying people's blood. They are likely to involve other genes that function in the synapse because so many of the genes we know are regulating the synapse.

And so, why are we doing this? Why do we want to understand the genetics of the disease? And I think really Charles Sawyers' talk illustrated that so beautifully for cancer. It's all about understanding the mechanism of a disease, so that people in your generation can hopefully develop better treatment for it. And the other, I think, paradigm shift that has come to this community, is our understanding that, in the autism brain, the structure of the brain seems to be mostly normal. The neurons seem to be in the right place, their axons seem to be mostly in the right place, they seem to be ready to act, but the difficulty is in the communication of one cell to another. And so, animal studies have suggested that some of the animals that have autism-like mutations can respond very well to chemical treatments that improve the synapses, even in adult mice, and so we actually now can start thinking about whether, if we could find good drugs that would regulate the synapse, might these be able to help children and even young adults that are on the autism spectrum.

In fact now, many places are setting up specialty clinics for some of the genetic forms of autism, specifically to do clinical trials, and there are clinical trials underway at several places to try to test drugs that will hopefully improve cognition and social behavior in kids on the autism spectrum. An example is a drug called arbaclofen, which regulates some of the receptors in the synapses, and there is evidence from animal models that this may well improve the function of the autistic synapse. In fact, there are other drugs that are repurposed cancer drugs, that regulate some of the kinases in cancers that also have important roles in the brain, and some of these cancer drugs are now being repurposed to test to see whether they might also improve the function of the brain of autistic kids as well.

And so we are very, very hopeful that, since autism seems to be a disorder of the synaptic plasticity and the synaptic change, and since we know that many autistic kids can respond and improve in response to environmental enrichment, we have a lot of hope that, as our mechanistic understanding improves, that in the future, I'm not talking about next year, but I'm talking about in the next years, that we might have a lot more to offer kids on the autism spectrum than we do today.

So I think I'll conclude at that point, and I welcome any other questions on the whole lecture. Yeah.

[STUDENT:] Is it possible, if the way that the brain develops, that if there were mosaic mutations in the synapses, that the mutations are localized in just areas of the brain that deal with social and communication as opposed to just like an entire half of the brain?

[DR. WALSH:] That's a fascinating question, and that is formally possible because the different parts of the brain like the amygdala that represents … the amygdala, for example, is very involved in emotional processing and fear. That's the sort of a structure that you think might be important to autism, and in fact, the cells of the amygdala are set aside from the rest of the brain at a relatively early age, so it's possible to imagine that a mutation might be more prevalent in the amygdala and less prevalent in the rest of the brain and might preferentially affect certain parts of the brain. Yes?

[STUDENT:] You said earlier in the first half of your talk that some children grow out of their social awkwardness. How likely is it that they will actually recess or, I guess, move down the spectrum and become worse?

[DR. WALSH:] So, that's not my particular area of specialty, but my sense is that some grow out and never go back, and then some might be sort of in and out over the years. But it's probably about 20% or so that are given a diagnosis of autism spectrum disorders at an early age and then grow their way out of it. And exactly how that happens, of course, is not known, and that's really a holy grail to see if we can make that number better. Yeah?

[STUDENT:] In cell communication between neurons in an autism patient, are the signals being transmitted in a different way than usual or just slower?

[DR. WALSH:] So there are a variety of ways in which those signals can be disrupted and it depends, to a certain extent, on which gene. So there are genes that can affect the presynaptic cell, right? The cell that was up in the upper left-hand corner of the diagram, and those cells normally have to release

chemicals that diffuse and affect the spine of the second cell, and so in those, if you affect the genes in that presynaptic cell, you might not release enough of the chemical.

On the other hand, the genes that act in the post-synaptic cell seem to regulate the response to the chemicals, and so you can get a variety of different defects; not enough released, or not enough response to the same amount of release. Yeah?

[STUDENT:] Is there evidence to suggest, or do you believe that there is an association between the severity of the mutation and the severity of the level of autism seen in the patient?

[DR. WALSH:] Yeah, that's a great question, and there is indeed good evidence that the children that have the lower functioning forms of autism, with larger co-morbidities like intellectual disability and seizures, are more likely to have a definable mutation, whether it's a large copy number variant or a point mutation as well. There are a number of studies that have suggested that. And it might be that … and in contrast, the kids who have the very high-functioning autism with well-preserved intelligence, they still do have definable genetic causes in a certain proportion of cases but a smaller fraction of the higher-functioning kids will have genetic causes. Yeah.

[STUDENT:] How is it possible that one out of two identical twins has a higher probability of having autism spectrum disorder as opposed to the other?

[DR. WALSH:] Well, there are definitely identical twins that are discordant for autism, and so that has been an area also of intense interest, to try to understand, to compare the genomes of those two identical twins, because you would think that that would get you to … there might be a single mutation that is present in the one identical twin and not in the other. So there are a lot of studies in progress, I can't think of a discordant set of identical twins where people have yet found a single genetic mutation that would explain that discordance, but I'm sure that people will eventually. Yeah.

[STUDENT:] I know that introns are considered junk DNA because they don't express proteins, but is it possible that the remaining causes of autism could relate to epigenetic disorders?

[DR. WALSH:] That's a … the epigenetics is a whole area that I didn't mention at all because we don't have that much firm data, but it's an area of an intense interest. There are certainly some causes of autism that are regulated epigenetically. There is a particular mutation that affects chromosome 15, which only causes autism if you inherit it from the mother, but if you inherit it from the father, it doesn't cause a problem at all, and that's because that particular part of chromosome 15 is only utilized on the mother's copy. And of course there are all sorts of ways in which genes are regulated epigenetically in a very complex way. Epigenetics is very important to learning and memory because actually learning things causes epigenetic changes in DNA, so there is a lot to think about in terms of epigenetics and autism spectrum disorders. But it is not clear that it's an identifiable cause for which we will have a diagnostic. In the back there.

[STUDENT:] In our theory of knowledge class, we have been having the argument that with the advancement in neurosciences, that the field of the human sciences will be changed or even

overshadowed with the type of diagnostic or work with people with neurological … such as autism and schizophrenia. Do you think that … do you see that happening now? Do you think that the human sciences as it is now will survive with these advancements?

[DR. WALSH:] Well, no I don't think that … you know, I think there's a … this ability of the same genetic mutation to cause different manifestations in different people. What that really means to me is that behavior has a genetic component, but it's not a simple one-to-one mapping. It's remarkable, I think, when you hear, in the dog talk, there are certain behaviors of dogs that seem to show a large genetic component. But for these things, it seems like certain mutations can impair the brain but in fact don't manifest themselves as a one-to-one cause of a particular disease. And I think that that, my own take on that, is that in fact, our huge cerebral cortex has a lot of different roles, but in fact, one of its roles may be to dodge genetics—that it might be this large plastic area where, in fact, … the reason why these different manifestations occur in different people might be because the formation of our brain, especially the cortex, is only imperfectly defined by our genes. And nature actually decided that they are going to give the human brain a large play space, where, in fact, learning and culture can have a bigger role. And so, I don't see any worry any time soon that we are all going to be subject to some sort of genetic determinism, that you'll be able to look at somebody's genome and say, oh yeah, you know, I know what kind of person that is, as though you've met him on the street. I think that's very unlikely to happen because I think the relationship is quite imperfect. Yeah?

[STUDENT:] Do you think that our quest to enhance ourselves will cause more problems than we are capable of handling?

[DR. WALSH:] Well, a simple, a short answer for that would be no. I think that we're very resilient people and that maybe I'm being blindly optimistic, but I have a lot of faith in our ability to handle knowledge. I think that we've done pretty well so far, at least when it comes to these sorts of challenges. I think that one of the beauties of doing science is that it allows you to sort of think about these big questions, and also it's nice to see how science can influence society, but I see a lot of hope and certainly I think that if you consider the balance of … should we push forward, the balance is very strongly in favor of pushing forward, because we can see in just a few years how we have a chance to do a lot of good, to try to help a lot of kids and families. Yeah.

[STUDENT:] Studies suggest that men are more likely than women to get autism. Why do you believe that is?

[DR. WALSH:] That is actually the most … that is probably the most fascinating question about autism and the most mysterious. Particularly high-functioning autism is about … males have about 9 times the risk of females of having high-functioning autism. As you go to the lower-functioning autism, there is still a male excess, but it's only about three-fold higher, and so, now that we understand some of the genetic mutations we can start thinking about that in a little bit more sophisticated way, and in fact we can see that some of these mutations have strong effects, like copy number variants, in fact have a higher penetrance in males than in females. And we can see the same genetic mutation is more likely to cause autism in a male than a female. So there is something about the male brain which is more

fragile to this particular thing, and it's anybody's guess at this point as to exactly why that would be, why that might be, whether that has to do with hormonal effects, whether it has to do with some essential differences in the wiring or differences in behavior. We don't really know, and I think that … or whether it's a difference in chromatin structure, where males have slightly different chromatin structure in their brain, and as I say it's a real mystery but a fascinating one. There's a woman behind you in the white.

[STUDENT:] My question is, I've heard people talk about the critical period, or the critical age, where between ages three or six is where children learn the most how to communicate, and if they don't learn in that period, they can never come back. What do you know about that and what's your view on that?

[DR. WALSH:] Yeah. Well, so early education is extremely important. Many, many studies have shown that. So that's why there's a big push to try to diagnose autism at the earliest possible stage so that we can try to intervene with the, with our existing treatments like environmental enrichment and intensive behavioral therapy, and so we're hoping that with the greater utility of genetics we will be able to offer than earlier diagnosis. Thank you very much.

[applause]

[music plays]