CURE Webinar
Epilepsy Genetics
(Transcript)

Dr. Laura Lubbers: 00:05

Welcome, everyone. And welcome to today's webinar on the first day of Epilepsy Awareness Month. My name is Laura Lubbers, and I'm the Chief Scientific Officer of Citizens United for Research in Epilepsy or CURE. And I'd like to thank each and every one of you for joining us today. CURE is excited to present this first in a series of five webinars, during Epilepsy Awareness Month. These will highlight some key research that is being done in the field of epilepsy. Today's webinar is being sponsored by GeneDx and B. Braun CeGaT, and will focus on the genetics of epilepsy.

Dr. Laura Lubbers: 01:02

CURE will also be hosting webinars on epilepsy devices and technologies on November 8th, featuring Dr. Robert Fisher from Stanford University. On November 15th, we'll have Dr. Jeff Noebels from Baylor College present on infantile spasms. And on November 21st, Dr. Ramon Diaz-Arrastia from the University of Pennsylvania will present on post traumatic epilepsy. Finally, during the last week of November on the 29th, Dr. Elizabeth Donner from the University of Toronto, will present on sudden unexpected death in epilepsy or SUDEP. All of these webinars are free and are at 2:00 PM Eastern Time US. We hope that you will join us for all of them.

Dr. Laura Lubbers: 01:49

For those of you who may not be familiar with CURE, our mission is to cure epilepsy. And transform and save millions of lives. We identify cutting-edge research, challenging scientists worldwide to collaborate and innovate in pursuit of this goal. We are one of the largest private funders of epilepsy research in the world, funding over 200 research projects in 15 countries. CURE has been the pioneer in many areas of epilepsy research including SUDEP, where we've invested over $4 million into research. CURE has also worked to accelerate the understanding of infantile spasms and find promising new treatments that may come into the clinic. We also realize that everyone is at risk for developing epilepsy because it can be acquired over time, and as a result of brain injury.

Dr. Laura Lubbers: 02:46

In fact, we've just launched a new project funded by a $10 million grant from the US Department of Defense that supports a team approach to understanding post traumatic epilepsy. CURE is also proud to contribute to research that is being done in the field of epilepsy genetics, the topic of today's talk. Many people don't know the cause of their epilepsy, but genetic research is changing that. Nearly three years ago, CURE founded the Epilepsy Genetics Initiative, or EGI. To help broaden our understanding of the genetic causes of epilepsy, and lead us

towards personalized medicine. EGI's centralized database is housed at Columbia University in New York City, and holds the genetic data of those with epilepsy. These data are analyzed and reanalyzed until the cause of the person's epilepsy is found.

Dr. Laura Lubbers: 03:38 Findings are reported back to the patient via their physician and the data are being made available in an anonymized way to help researchers learn more about epilepsy. The EGI database continues to grow and currently has over 700 participants with nearly 20 institutions from around the world directly contributing data. EGI has identified a diagnosis for 10 individuals who previously didn't have a genetic diagnosis and has implicated a new gene responsible for epilepsy. EGI has proven to be a valuable resource for the epilepsy community and we are excited to see how these new diagnoses and research findings may lead to new or improved treatment options for patients. Today, we have Dr. Dan Lowenstein, who will be talking to us further about the genetics of epilepsy. Dr. Lowenstein is based at the University of California, San Francisco. Where he is the Executive Vice Chancellor and Provost, as well as the Robert B. and Ellinor Aird Professor.

Dr. Laura Lubbers: 04:43 He also serves as the Vice Chairman in the Department of Neurology, and as the Director of the Epilepsy Center. Dr. Lowenstein received his bachelor's degree in mathematics from the University of Colorado, a master's degree from the Pennsylvania State University and his medical degree from Harvard Medical School. He's been recognized nationally and internationally for his research and commitment to the field of epilepsy. And recently was elected to the National Academy of Medicine, which is among the most prestigious honors in the fields of health and medicine. Before Dr. Lowenstein begins, I'd like to encourage everyone to be thinking about and prepared to ask questions. You may submit your questions anytime during the presentation by typing them into the Questions tab of the GoToWebinar control panel, and clicking Send.

Dr. Laura Lubbers: 05:33 My colleague from CURE, Brandon Laughlin, will read them aloud during the Q&A portion of the webinar. We really do want this webinar to be as interactive and informative as possible. However, to respect everybody's privacy, we ask that you make your questions general and not specific to a loved one's epilepsy. I also want to mention that today's webinar, as well as the entire Leaders in Epilepsy Research Webinar Series will be recorded and available on the CURE website. So with that, I'd like to turn it over to Dr. Lowenstein. Dan.

Dr. Dan Lowenstein: 06:07 Thanks so much, Laura. And welcome everyone to this session, which is as you just heard the first of a whole series during this month. And I want to wish you all a happy Halloween and November 1st. And good morning or good afternoon or whatever time of day it might be for you where you're checking in on this session. And I also want to highlight what Laura said previously about the work that CURE has been doing. CURE has been one of the leading lights in the world, recognizing the vital importance of doing basic and translational research in order for us to better the lives of people living with epilepsy. And as someone who has been involved directly in the research enterprise over pretty much my entire career, and have also had the opportunity to take care of patients and their families living with epilepsy,

Dr. Dan Lowenstein: 07:08 I am as passionate as can be about the importance of doing basic and translational research in order for us to advance our understanding of the basis of epilepsy, because that is key to improve therapies and eventually accomplishing cures. So with that, let me introduce you to the plans for this session. What I'd like to discuss with you over the next 45 minutes or so, is the nature of epilepsy as it relates to our genes. And as many of you undoubtedly know, genetics is a field of study that relates to the connection between our genes and the illnesses, and states of health that we all experience. So, to begin, there are many causes of epilepsy. And as shown in this figure, genetics presumably accounts to the majority of the causes of epilepsy. Roughly two-thirds of all causes.

Dr. Dan Lowenstein: 08:41 And as you can see, other important causes are those related to congenital abnormalities during the formation of the fetus. Traumatic brain injury, strokes or ischemia to the brain. And then there are many, many other subcategories. But it has been recognized for a very long-time that genetics is one of the most important factors. Evidence that came out a few decades ago was based on population studies. And in this particular figure, I'm showing you some data that came from a very large population based study out of Minnesota. In which the population was studied of those who did and did not have epilepsy. And for each individual who was the main focus of attention for having had epilepsy. The investigators then asked if we look at the other family members. Whether it's siblings, brothers or sisters, children, if there were parents, nieces and nephews, grandchildren and so forth.

Dr. Dan Lowenstein: 09:56 There was a certain number of observed cases shown in the first column. And that was compared to the number of expected cases based on other normal population data. And the key

statistic here is what's called the relative risk. Which is highlighted here within the red oval. And as you can see the relative risk of a sibling or a child, in particular, for someone who has epilepsy was significantly higher than the general population. So this is clear evidence of the role of genetics in epilepsy broadly defined. Now, what I'd like to do over the next 10 or 15 minutes, is really drill down and focus on the basics of genetics itself. So I'm calling this Genetics 101. And I'm assuming that there's a fair range of understanding about genetics among all the people who are calling in on this webinar. And I just decided I'd really go to the basics, because it's important in order to understand how genes might ultimately affect the predisposition for epilepsy to have the basic concepts of genetics in your mind.

Dr. Dan Lowenstein: 11:15 So I apologize for those of you where this might be too basic. But I'm hoping that there are a number of you that will find this quite informative. So first off, I think everyone recognizes the fact that our body is made up of many different types of cells, billions upon billions of cells. In fact, it's the cells and then water which pretty much make up who we are. So our cells come in many different types depending on the organ. And as shown in this figure, if you look at a particular organ, the cell will have a certain set of characteristics. It will have a unique morphology, it'll look a certain way. And of course, that is an indicator of the many different types of functions that our cells have. So for example, heart cells are designed in a way to contract in a synchronized way to allow a pumping action of the heart.

Dr. Dan Lowenstein: 12:15 The kidney is, has a whole variety of different cells which also ultimately serve as a filter of our blood. But the focus of attention, of course, today is going to be on the cells that make up our brain. And these are a variety of cells dominated in terms of functionally by cells called neurons, as well as other cells called glia, which offer more of a support structure to the brain. So if we now take a look at an individual cell, and this cartoon is meant just to give you a generic idea of what a cell looks like. The blue outward envelope is the cell membrane, which keeps in the contents of the cell. And I think it's fair to say that within the world of an individual cell, there's actually there's a universe in itself within a cell.

Dr. Dan Lowenstein: 13:34 And some of the components are shown here. Structures called ribosomes and the source of energy for the cell, mitochondria. But again, the focus for today is going to be on this structure shown near the center of the cell, which is called the nucleus. And the nucleus again, as I'm sure most of you realized is the place that contains the DNA or the code for the architecture

and the function of our body. Now let's drill in even further, if we look inside the cell nucleus, and I'm going to highlight this one more time. Again, here's within the nucleus. And here is one of a number of chromosomes that exist within the nucleus of the cell. So now let's focus in on the chromosome itself. Well, the chromosome is made up of DNA or the double helix shown here. And this double helix is wrapped and twirled among itself with some other proteins to ultimately become what are called super coils, which then are packed into individual chromosomes.

Dr. Dan Lowenstein: 14:49

So you should have an appreciation that a chromosome is made up of millions upon millions of strands of DNA. Now it turns out that we have, humans have 23 chromosomes. 22 of which are shown here, which have to do with many of the ... all the characteristics that make up our cells. And then two special ones called the X and the Y chromosome, which are the ones that determine our sex and carry other genes related to that. So this is what chromosomes look like within a nucleus. Now let's look a little bit further. And consider how the various chromosomes determine what we become or the term is called our phenotype. The phenotype is the characteristics that are a result of the particular genetic makeup that we have. And here I'm taking one of the simplest cases in how the chromosomal material from the sex chromosomes determine our sex. So the father provides a chromosome that is either Y or X. And if I go back, I'll highlight that here. It's either the X chromosome or the Y chromosome.

Dr. Dan Lowenstein: 16:15

So the father can contribute one or the other, and normally only one of those two. Whereas the mother only contributes X chromosomes, shown here. And depending on chance if the child receives a Y chromosome from the Father and an X chromosome from the mother, that child will be a male. Whereas if the father happens to contribute an X chromosome and the mother an X chromosome, the child will be a female. And again, this is probably the simplest example of how the contributions from the chromosomes from the mother or the father, ultimately characterize the phenotype of the child. And for every trait that we have as humans, these are determined by the chance contribution of the chromosomes from the mother or father, along with a much more complicated part of the process in which there is exchange of genetic material between the chromosomes. But I won't go into that in any detail.

Dr. Dan Lowenstein: 17:19

Now, let's talk a little bit about how the DNA code ultimately determines our makeup. And it does this by actually a remarkable system, which was essentially discovered back in

the 1950s after decades of work. Which has shown that for the four different types of DNA bases, and we can use the letters A, G, C and T. It is the particular sequence of three of those bases that determine the genetic code. So you can see in the top part, this is just taking a stretch of DNA. Seven, so called base pairs from this DNA helix. And the code is, in this case A, C, G, T, A, C, C. And it continues on for billions of base pairs in our, the chromosomes within our nuclei. And these match up in a very specific way. A's go to T, C's go to G's. And you'll see that this matching is consistent all across this stretch of DNA. And what's so interesting is that depending on this triplet or these three letters that are in sequence, that is the determinant of a particular amino acid.

Dr. Dan Lowenstein: 18:38

And amino acids are more complex structures that are the building blocks of proteins. And proteins are the molecules within our bodies that determine structure and form. And I pulled this off of the web, this figure. Because I really liked the way the artist chose to use Lego blocks as the different types of amino acids. And so you can see that this particular sequence ACG encodes for a particular protein, that's called threonine. So it's a green small Lego. Whereas these three, TAC encode a completely different amino acid called tyrosine. Which would be one of those longer, in this case, white Lego blocks. And it is the sequence and the joining together of these different molecules, of these different amino acids that ultimately make the more complex structure of a protein. And here are just two examples of proteins made up of Lego blocks. This one happens to be a protein that we might think has something to do with airplanes and flying.

Dr. Dan Lowenstein: 19:48

And here's a more complex structure. But I hope you can appreciate that it is the sequence of the triplets within our DNA, this coding that determines the sequence of the amino acids that ultimately form these more complex structures. And the level of complexity is phenomenal. And you can imagine, in this very complex part of a Lego City on how this was determined. Again, by millions upon millions of triplets of DNA coding that has specified each individual building block and created complex structures like whole buildings, or harbors, or train tracks and so forth. And this ultimately is what determines the nature of each individual cell in our body, and therefore the organs and ultimately our entire phenotype. Now, let's ... with that understanding, I'm assuming that you're following me here. And you have a sense of how cells make up the body, how within cells there are nuclei, within the nuclei are chromosomes.

Dr. Dan Lowenstein: 21:10

Chromosomes are made up of DNA, the sequence of the individual bits of DNA determine which amino acid is going to be connected to the next amino acid. And it's that connection of amino acids that ultimately determine the proteins, which define the structure and function of ourselves. If you have that all, if you have that conceptually in mind, now you're ready to really understand how mutations in DNA cause abnormal proteins. Now, this figure is complicated, but let's walk through it. What's shown here at the top is a sequence of DNA triplets. Again, these are the determinants, this is the genetic code in each of our bodies, and it's unique for each one of us. In this particular stretch of four triplets, we happen to have the sequence AAA, then ATG, CTT and CTC.

Dr. Dan Lowenstein: 22:10

And as you'll recall, each of these have a specific match to other nucleotides. In this case, I'm showing something called mRNA, which is messenger RNA. Which is what ultimately allows us to create our sequence or chain of amino acids. So the pairing is very specific, you'll see that A always goes with U. T always goes with A, G with C, C with G. Here's another T with A, T with A and so forth. So that is the normal encoding. And these are the particular amino acids that are determined by these sequences. So if you have a UUU it creates this amino acid called phenylalanine (PHA). If UAC it makes tyrosine, and so forth. And there are 20 of these amino acids. Now to make this a little bit clearer, I decided I would use the Lego analogy and replace those amino acid names with different shapes of Lego blocks. So in our normal DNA shown here, the sequence is determining that the DNA AAA encode UUU which creates a long rectangular blue Lego block.

Dr. Dan Lowenstein: 23:41

As you can see, UAC creates a small square yellow block. GAA makes a long rectangular green block. And it turns out that there's redundancy in the codes. And so there are some times where two different triplets actually encode the same amino acids. So that's why I show another green Lego block here. So I hope you can understand how this sequence of DNA is leading to this chain or necklace of amino acids, which happen to have this sequence. All right, now let's take a look at how mutations or changes in the DNA can cause abnormal proteins. So let's focus in on a nuclei, on a change that's called a substitution. A mutation has been introduced into the DNA in one particular area, and if we follow the code here, and compare it to the normal code here, you'll see that everything looks correct until we get to here.

Dr. Dan Lowenstein: 24:52

And what we have then is a nucleotide, the nucleotide T has replaced the normal nucleotide C. And this means, we'll talk a

little bit later about how these mutations might arise. But this could be inherited from one of the parents. It could be caused by some kind of environmental effect that's created a mutation in the DNA. Or it can arise new in the developing embryo or fetus. And with that's a term that we'll come back to called de novo. De novo or new. But regardless of the reason, I hope you can appreciate that this normal sequence of DNA now has a mutation in it. And the mechanism of this mutation was a substitution of a T for a C. Now that creates a new triplet. So instead of encoding CTT, which normally creates a green long rectangular Lego block.

Dr. Dan Lowenstein: 26:00 We now have instead of GAA we have AAA, which encodes for an entirely different amino acid, a long rectangular red Lego block. And now look, instead of having this normal sequence within this protein, we have an abnormal sequence. And because of this, this protein is going to be abnormal in its structure and function. Now to be actually more precise, it turns out that changes in amino acids don't necessarily cause a damaging mutation. So we actually do have a fair number or lots of variation in our DNA, which leads to these types of substitutions. But fortunately, doesn't necessarily lead to a protein that will not function normally. But for the purposes of our discussion, I'm going to focus in on the fact that when these changes in our DNA occur, they lead to the possibility of having an abnormal protein.

Dr. Dan Lowenstein: 27:09 Now at this point in my presentation, I realized I can't get any feedback from you directly right now. But I'm really hoping that you're following me along. Because this is going to be so helpful, I think in your understanding of the inheritance of the epilepsies. I'm going to describe two other mechanisms for mutations causing abnormal proteins. Here, take a look at the DNA sequence. Again, we have the normal sequence here AAA, here's ATG, just like here. But now we have an abnormal insertion of a nucleotide that wasn't there before. So as you'll notice, a C has been newly introduced into the sequence. It's inserted itself between this G and this C. And these subsequent nucleotides have been shifted over downstream, shown here. So the new nucleotide is here. And now we have CTT which used to be a normal encoding of an amino acid, but it's been shifted.

Dr. Dan Lowenstein: 28:20 And so this alters the sequence. So now instead of having CTT, we have CCT, which as you can see encodes a different amino acid. In this case, a long rectangular blue Lego. Whereas it should have been green. And furthermore, because of the shift in the sequence, we no longer have CTC here, we actually have

TCT because of the shift, and we have yet a new amino acid. So you can see now how different our amino acid string or necklace looks compared to the normal encoding. Finally, here's an example of a deletion. So you'll know the normal sequence is AAA and then ATG. In this case, the fourth A here has been removed, it's gone. And now we have a shift to the other direction of all the downstream nucleotides. So instead of then going to ATG ....

Dr. Dan Lowenstein: 29:27 Instead of going to ATG, because of the loss of this A things have been shifted over to the left. And we now have a triplet that is TGC, which encodes a different amino acid. So instead of the normal small square yellow Lego, we have a light green rectangular one. And this is actually now shifted the other downstream amino acids as well, and you can see how abnormal they are. So we have a completely different amino acid sequence. All right. Now I hope you have an understanding of the different ways mutation in DNA can ultimately lead to changes in protein. So now let's look at some of the different ways that DNA mutations can ultimately appear in a child. This figure shows what's called autosomal dominant inheritance. What this means is that among the chromosomes that are provided from the mother or the dad, and remember each one gives one of the two chromosomes that they have among those 23 pairs.

Dr. Dan Lowenstein: 30:31 In this case, the father has one of the chromosomes with an abnormal copy of the gene. In other words, it has one of the types of mutations that we've just been talking about. And depending on by chance, which chromosome is given by the mother or the father. In this case, this child received two normal chromosomes, this one and this one. Or this one, and this one, but both normal. So this child was normal. Another child, also normal. But in this case, this child or this child received a normal chromosome from the mother. But because the chromosome from the father has the mutation, the child is affected because it's so called dominant. In other words, if you have one copy of the mutation from either of your parents, you will have the disease associated with that mutation. Now, you can contrast that to what's called autosomal recessive inheritance.

Dr. Dan Lowenstein: 31:34 In this case, the mother has one chromosome that's normal and one that's abnormal, and so does the Father. So depending on which chromosome comes to the child, that individual will be affected either by having one chromosome, in which case we call that person a carrier. So that they may not have the disease because they only have one abnormal chromosome. But in the case of the child who by chance receives the abnormal

chromosome from the mother and the abnormal chromosome from the Father, that child will be affected because having both mutations in each chromosome leads to the disease. Now, there's one other mechanism that within the world of genetics and in particular, the world of epilepsy has become quite important. And that's what I referred to before, which is called a de novo mutation.

Dr. Dan Lowenstein: 32:30

And in this case, the father's chromosomes are completely normal. The mother's chromosomes are completely normal. However, within the child during the first formation of the child, a mutation arose either in the sperm of the father or the egg of the mother. So it's not present in all the other cells in the parents body, but in the sperm or the egg a new mutation appears. And that mutation then was part of the developing embryo and fetus, and ultimately the child. So we call this as I said before, a de novo, a new mutation that is not inherited from the family. It arose during the formation of the offspring. Finally, I'd like to come back to the cells of the brain. And as I said at the outset, our brain is the most extraordinary organ. I tell my medical students, I think it's the most remarkable and complex entity in the known universe. Our brain is made up of many different cell types, one of which are the neurons.

Dr. Dan Lowenstein: 33:52

And neurons are designed by nature to be signaling machines. They send off electrical signals and make billions upon billions of connections with other cells in the brain to ultimately allow us to have the functions of the nervous system. So neurons are designed to send off signals, and they do this by changes in electricity. The main way that neurons alter their electrical properties is by allowing for the passage of ions through these proteins that are called channels. And depicted here are individual ions, these little purple dots on the outside of the cell this is the membrane of the cell. The nucleus would be way down inside and the rest of the universe of the cell that we looked at a few slides ago. And what I'm trying to show here, in the artist's rendition is to appreciate that all these different ions floating on the outside of the cell can pass into the cell through this pore or this channel within the protein.

Dr. Dan Lowenstein: 35:05

And it's the passage of these ions that determine the electrical properties of our neurons. Now here's an example, I've altered this artist's rendition slightly, so look at it carefully. But I hope you can appreciate that I've depicted a mutation in this so called ion channel. That has allowed a widening of the channel itself or the pore. And because of this, there's more passage of electrically charged ions through this channel into the cell. So there are now more ions on the inside of the cell than would

normally occur. And I hope you can appreciate that this alteration in the passage of these electrically charged ions would change the fundamental properties of the firing off of the electrical signaling of these individual neurons in our brain. And this is one example of how mutations in proteins can cause epilepsy, by altering the channel properties that determine the electrical firing in the neurons within our brain.

Dr. Dan Lowenstein: 36:20 And this brings me to my last two slides. Over the last 30 years or so, we've seen an explosion in our understanding of the genetic basis of epilepsy. And one of the very first papers came out in 1989. And it was a study of a family with a very complex family tree. But all the circles or squares in black represent individuals with a certain type of epilepsy. And it was by studying families like this, that the first epilepsy genes were discovered. And this final slide gives you a sense of the genetic landscape now in 2017. Basically, we now know that there are relatively rare families less than 5% that have the more simple type of inheritance that I talked about before, autosomal dominant, autosomal recessive. And we know that single genes are the cause of those epilepsies, gene mutations are the causes of those epilepsies.

Dr. Dan Lowenstein: 37:21 Another example, are the epileptic encephalopathies. And Jeff Noebels is going to be talking about infantile spasms in a couple weeks. We now appreciate that many of these are due to the de novo mutations that I've covered in some detail. And finally, we know that the majority of the cases have a much more complex inheritance. We're still at the beginning of being able to understand the many genes that contribute to this. Recent papers have identified some of them, and they overlap with the genes that have been found in these other rare forms. But a significant amount of progress is being made to date. So with that, I'll end my slide presentation and turn to Brandon for some of the questions that you might have.

Dr. Laura Lubbers: 38:08 Thank you, Dan. As Dan mentioned, we can now begin the Q&A session. Again, if you do have questions please submit them in the Questions tab of the GoToWebinar control panel and click Send. And Brandon can go ahead and start reading them aloud. I know we already have some questions. Brandon, take it away.

Brandon Laughlin: 38:26 Sure, thank you. First question was regarding what are the different types of genetic tests that are out there right now?

Dr. Dan Lowenstein: 38:42 Great question. The most common test involves taking a taking DNA, taking a blood sample and applying it to what we call an array. An array is essentially a matrix that includes a panel, if

you will, of the most common types of genes that have thus far been associated with various genetic forms of epilepsy. So a DNA panel will include, whether it's 50 or 100, or 50 or 500 genes in which there is evidence that there is an association with epilepsy, and it will test specifically for those genes. On the other end of the spectrum is called, is something called whole exome sequencing. Now, if I use the term whole genome sequencing, I think most of you would probably appreciate that that means that the process involves sequencing our entire genome. The three billion base pairs of our genome. But it turns out that only a very small percentage of our genome actually encodes proteins themselves. And that part of the genome that encodes proteins is called an exome.

Dr. Dan Lowenstein: 40:08

So whole exome sequencing is much less costly and much more efficient. And so we're turning more and more to doing whole exome testing so that we have a chance to, I say, the entire exome of an individual rather than just being limited to the DNA panels or arrays. These are carried out by clinical testing labs all across the country and in the world now. And the main limitation at this point for people in which there is a reasonable chance that a genetic cause might be identified. The real challenge that we're currently having at the moment is getting health insurance to cover the cost of that testing. You heard from Laura before that the epilepsy genetics initiative is a large national, international effort to try and collect as much of the data that is being produced in these clinical testing labs as well as research labs. So that we can deepen our understanding of the complexity of the exome and discover more and more epilepsy genes.

Brandon Laughlin: 41:20

Great. Next question. Can you speak briefly about some of the environmental factors that can actually modify genes?

Dr. Dan Lowenstein: 41:30

Yeah, we have a fairly limited understanding of that to date. I think the most obvious environmental factor that we know can mutate DNA is actually sunlight. So we know that ultraviolet exposure and extreme sunlight can directly damage DNA and the evidence for that is that as we age, we have more and more of a predisposition for skin cancer. Especially any of us who have not taken a caution on using proper protection lotion throughout our life. So light itself is directly damaging. There's also evidence, clear evidence of various toxins to be damaging to DNA, so called teratogens. And in some cases that's even been associated with certain drugs. We know that a very significant number of environmental toxins associated with pesticides can be DNA damaging as well. But this is an area that I think has received relatively limited study to date. And my

hope is that we're going to make significant advances in the years ahead in that regard.

Brandon Laughlin: 43:04 Great. Next question. Can you just clarify again, some about how a child can inherit epilepsy from a parent and the parent be unaffected, even though it did come from them?

Dr. Dan Lowenstein: 43:18 Yes. So in the most well understood case, the parents may be unaffected, but there may be other members of the family that are affected. So that goes back to the family tree that I showed before. So in that case, if the parent is carrying one copy of the gene that person won't be affected. But if they happen to have a child with a partner who also carries that gene, then the child would receive both copies and be affected. So that's the most straightforward case. But I think the questioner is asking what about if there's not any epilepsy that's evident in the family? And especially in the case where you can really trace the family tree on both sides. And that individual may be carrying a mutation that is not causing epilepsy. But the child, in fact, does then develop epilepsy.

Dr. Dan Lowenstein: 44:20 We don't completely understand this yet. But that gets back to the previous figure, where I showed that we believe that many, many cases in which there's a genetic influence on the epilepsy is due to polygenic changes or small change, relatively small changes in the various proteins. But many proteins because a number of different genes are affected, so it's not a single gene mutation. Of course, the other very important reason for a child developing epilepsy born today to both parents who don't have any evidence of epilepsy is what I talked about before. And that's this idea of de novo mutations. In other words, the mutation in the child was not inherited from the DNA from the parents, it appeared only in the sperm and the egg. And so this is not something that would be inherited. Now, that child now does carry that mutation. And if that child had children, depending on the mutation and severity, it could theoretically be passed on to the children of that particular child.

Brandon Laughlin: 45:37 Great. Next question, if EGI has a population of 700 plus participants, how many are needed to do an effective population level genetic analysis of epilepsy?

Dr. Dan Lowenstein: 45:50 Oh, that's a beautiful question. So if you can go back to the previous slide, Brandon or Laura, just to bring that up again. I just want to emphasize this again. The vast majority of cases of people who have epilepsy, in which there's a genetic, a primary genetic cause or genetic influence, have that epilepsy because of sometimes subtle mutations in many, many genes. The only

way that we're going to be able to figure this out is by doing a genetic analysis by doing sequencing, whole exome and whole genome sequencing on hundreds of thousands of people with particular, with epilepsy or whatever the disease type it is. We now are appreciating just how complex the genetics is in the majority of cases. And we now believe that it's going to take certainly many, many, many tens of thousands of people. And ultimately I believe it's going to be hundreds of thousands of people for us to really decipher this.

Dr. Dan Lowenstein: 47:03 So EGI is a kind of approach that we need to have, where we bring together the different data-sets that are being produced all around the globe. Again, whether it's from individual clinical testing labs or large research groups that are doing sequencing on hundreds of people or thousands of people. If we can work out the arrangement to be able to bring all that data into one common data set. I don't have any question that we have the capacity for being able to do an analysis on hundreds of thousands of people from around the world, which is going to be the critical step for understanding the complexity of this type of genetic influence on epilepsy.

Brandon Laughlin: 47:51 Great. And this question is actually dealing with EGI and other research initiatives as well. But the question is, does the genetic testing have to be done with just the person who has epilepsy, or does it have to include other family members' DNA as well?

Dr. Dan Lowenstein: 48:09 Yeah, again, wonderful question. In many cases, it's the ideal is to have the DNA from the affected person and both parents. But this is actually changing, in part because of the creation of larger and larger data sets. The statistical geneticists are working out approaches that allow a comparison between just a single individual and the increasingly large data sets that contain sequence from both affected. And in our case, epilepsy patients. And very, very large, quote, "normal population controls". So at the moment, again, depending on the situation, the clinician ordering the test will prefer getting DNA from the affected person and both parents. But I think that this is changing and over time, just getting individual DNA will be sufficient.

Brandon Laughlin: 49:23 Great. Next question, what percentage of epilepsy cases do you expect that will be identified by whole exome sequencing in the future?

Dr. Dan Lowenstein: 49:36 Well, that's a tough one. It's a fool's errand to predict the future in this way. But, I mean, given that we believe that roughly two-thirds of all the epilepsies have some type of genetic influence.

And, by the way, I should also say that we think that in some cases of so called acquired epilepsy, that you're genetic makeup also helps determine your likelihood of developing epilepsy. For example, everyone probably appreciates that traumatic brain injury, such as what can occur in a terrible car accident can lead to epilepsy. And in that case, we believe that the main event is the fact that there's been a direct injury to the brain. But there is evidence to suggest that that individual's genetic makeup may make it more or less likely. Just depending on their, the nature of their DNA. So in those cases as well, I think that we'll get to the point where whole exome sequencing will allow us to essentially define what the map is of variations in DNA or mutations, that are the determinants of a person's disease.

Dr. Dan Lowenstein: 50:54

And I'm not going to say that it's going to necessarily be two-thirds of all patients with epilepsy, but I would think that conservatively that it'll be 50%. And there will be a day when we're going to have an entire map of our own DNA with the predictions of how various mutations might affect our likelihood of developing epilepsy. But more importantly by having that map, we will be able to come up with very specific precise approaches to therapy that are tailored to the specific genetic basis of that person's epilepsy.

Brandon Laughlin: 51:35

All right, thank you. It looks like we have time for about one more question here. And for those of you that asked about how to get involved in EGI, Laura will be giving you some contact information here in a few minutes that can help you out as well. And the last question is, is it more difficult to determine mutations in generalized ability given that the brain is firing neurons from all over?

Dr. Dan Lowenstein: 52:04

Great. That's a really, really good question. We have not figured that out yet. I've had the good fortune of being involved in a very large study called Epi4K that has involved hundreds of scientists and study coordinators throughout the world, in which we've collected and sequenced many thousands of patients with various forms of epilepsy. And we actually have attempted to answer that very specific question of what's the likelihood of finding a causative mutation in patients who have generalized epilepsies versus those with focal epilepsies. And somewhat to our surprise in our first main study, we were able to identify a larger number of causative mutations in the focal epilepsy. So that actually went against our hypothesis and I think what the questioner is asking. But I don't think this chapter has come to a close yet. I think there's a lot more research that knows, that needs to go into this. And I suspect

		that we'll be making advances in identifying mutations in both cases.
Brandon Laughlin:	53:19	Great. And Dan I'm actually going to address one more question, because it actually has popped up a couple times now. But is it possible for an individual to have a known genetic mutation, but not actually have the disease?
Dr. Dan Lowenstein:	53:35	Yes. So I alluded to this before, I didn't emphasize it. But all of us are walking around with variations. There are mutations, there are differences in DNA from one another. And some of us have variants that really are quite different from the normal population. And yet for reasons sometimes that we do and sometimes don't understand that variation does not lead to any significant abnormal structure or function of the protein. Or it may lead to an abnormal protein but nature has come up with some other ways of covering the deficit that might occur from that protein. And so we have our normal health. So yes, we're filled with variations in our DNA that fortunately more often than not actually don't cause disease.
Brandon Laughlin:	54:32	Great. And that will conclude the Q&A portion of today.
Dr. Laura Lubbers:	54:40	Terrific. Thank you both Dan and Brandon for fielding those questions. So as Brandon mentioned, this does conclude our epilepsy genetics webinar. I want to thank everyone for joining us today and giving a special thanks to Dr. Lowenstein for sharing his vast knowledge of epilepsy genetics in a way that we can all understand it. I'd also like to say to thank the teams at GeneDx and B. Braun CeGaT for sponsoring today's webinar. If you do have questions about EGI, or any of CURE's research programs, please visit our new website at www.cureepilepsy.org. That's one word, cureepilepsy.org. Or you can email us at info@cureepilepsy.org.
Dr. Laura Lubbers:	55:25	You can also find more information on CURE events during Epilepsy Awareness Month, as well as our My Shot at Epilepsy campaign on the website. And thinking about next week, please be sure to register for our next webinar. Which will be on November 8th, at 2:00 PM Eastern Time US. Again, Dr. Fisher from Stanford University will be joining us to discuss the latest research in the field of epilepsy devices and technology. Another very hot topic. So thank you again, all. And enjoy the rest of your day.