SPEAKER 1: So it's my real pleasure to introduce our speakers for this afternoon. First of all, Dr. Gianrico Farrugia, who I think virtually all of you know. He is the director for the Center for Individualized Medicine here at Mayo. Dr. Farrugia has done extensive research in gastrointestinal physiology. He's editor of the journal *Neurogastroenterology and Motility.*

His primary research interests are in gastrointestinal motility. He's published more than 200 articles, is extremely well-funded by NIH, and over the past several years has really led the Center for Individualized Medicine, which is really gaining not only institutional but national and international recognition for the work that they're doing in genomic medicine and personalized medicine. Our second presenter is Dr. Arthur Beyder. He is a fellow in the GI and hepatology department.

He grew up in the Ukraine and emigrated to the US in 1989. He graduated summa cum laude from the State University of New York in biophysics and mathematics. He then came to Mayo for further training. He joined the CI program through the Graduate School of Medicine and he is currently a fellow in GI and an assistant professor. His research interests also are in GI motility and he's work with Dr. Farrugia since 2007.

He's published numerous papers in high level journals, including*PNS, Circulation,* and *Gastroenterology.* So this is a combined presentation. They're going to talk about their work on specific aspects related to undiagnosed diseases and it's working differently with undiagnosed diseases.

GIANRICO FARRUGIA: I always have to start by making this a left-handed presentation. So thank you. I'll first go through the obligatory first two slides of disclosures-- and I suspect I need to do this. Oh, they did it. All right. And learning objectives. So welcome. Thanks for coming.

So Art and I are really glad to have this opportunity to present because this is something that has for us been about 16 years in coming to get to the point where we are today. And what I thought I'd do is just spend very brief 30 seconds making five points before we get into the meat of the presentation itself. And the first I just made, which is sometimes things take time. And this was a perfect example of one of those occasions that it's really taken us as I went back and looked at the first time recorded the sodium current and recorded the sodium current we're talking about 16 years ago.

The second point I want to make is really that when we started looking at this, this was discovery science. We saw a sodium channel that we had absolutely no clinical relevance for, and it was only that it seemed interesting enough that we wanted to devote some time to it. And sometimes, that's what you have to do. You have to go after leads that interest you. And sometimes they don't pan out and sometimes they do, and that's part of the process.

The third is obvious, but it's worth mentioning, is that this really is an only at Mayo Clinic story. Very quickly, we were out of our depth. We had to work with my colleagues in gastroenterology in the motility division. We also had to work with cardiology. And there are certain things that were totally outside of our expertise that we got help with very, very quickly and readily, including access to resources that would be very hard for me to figure out that we could have done it somewhere else.

But the fourth point to make is really the global aspect of it. Despite all that, there are 16 papers that got published before the last paper that got published a week ago. And that last paper required us to go and look at cohorts across the globe. So we had to collaborate with four different countries, numerous investigators. And it's really now symptomatic of team science is the ability to have to compromise and have to work with other people that don't work exactly in your area of expertise. And it's something that we need to keep on doing at Mayo.

The last, and why we're here today, is that this couldn't have happened without CCAT. We wanted to do an n of 1 clinical trial, and there's no way we would have been able to do without the resources and the infrastructure available. So we're grateful for that. So here's what I want to talk about. I want to level set by talking about irritable bowel syndrome, talk about ion channels in IBS, and then Art will take over and talk about specifically mutations in this channel and their link to irritable bowel syndrome.

So as I said, level setting-- I know most of you know, but it's good to make sure we're all talking about the same thing. Irritable bowel syndrome is clinically defined by a set of criteria that are known as Rome III. And it's recurrent abdominal pain or discomfort that lasts at least three days per month in the last three months. But it's not abdominal pain, that would be chronic abdominal pain. It has to be associated with a change in stool.

And you need to have at least two or more of the following, an improvement with defecation, onset associated with a change in frequency of the stool, and onset associated with a change in the form of the stool. And that's what makes irritable bowel syndrome different from chronic abdominal pain. Now, it is very common and it's also expensive.

So in the Western world, about 15% to 20% of the population meets the criteria for irritable bowel syndrome. That makes 45 to 60 million people in the US that have irritable bowel syndrome. And it's about 1.5 times more prevalent in women than it is in men. It carries with it a higher economic burden. So it accounts for about 30% of referrals to gastroenterology, it accounts for about 3.6 million visits in the US per year, and it accounts for about $20 billion in direct expenditures.

In fact, somebody with irritable bowel syndrome consumes about 50% more resources than somebody without irritable bowel syndrome. And it carries with it also an impact in the workplace. So people report that they're absent for about 5% of the work week and have impaired productivity for 30% of the workweek. Now, of course, something as broad as this is unlikely to have one cause and is likely to affect only one target, and that's certainly the case for irritable bowel syndrome.

We've learned over many years that there's an intense interaction between the brain and the gut. And of course, signals that are produced in the gut have to be processed by the brain and that plays a significant role in symptomatology. But we've also learned of specific abnormalities at the level of the gut itself. One of them is this concept of visceral hypersensitivity that is an increased in response to a given stimulus.

The second is that there are really well-defined changes at the level of the gastrointestinal tract when it comes to motility. Michael Camilleri has shown that at least 30% of people with IBS have a defined change in gastrointestinal motility. More recently, we're beginning to understand that there are changes at the level of the intestinal microbiome. Work done across the globe, but certainly including here with [INAUDIBLE] and [INAUDIBLE] leading the way. We've also recently learned that in irritable bowel syndrome, there's a change in epithelial permeability, work that Madhu Grover is doing here that can result in exposure of the inside of the guts to substances that it normally would not see.

And we've learned, and in fact, there are many drugs that have been designed around this concept, that's 5-HT and 5-HT-related targets are central in IBS. But we also know that IBS is a complex polygenic disease, and we know that from some of the work that suggests that it aggravates in families. Relatives are about two to three times at risk for IBS compared to non-relatives. Twin studies support this association. It affects multiple generations, but it's certainly not a Mendelian disease.

It is a complex genetic disease with contribution of either many variants that come together or rare variants that come together in unique combinations to predispose you to IBS. And when you summarize the literature, these are the genes that have been linked to irritable bowel syndrome. Some of them are stronger linked than others. What we are going to be speaking about today is a specific channel, which is a voltage gated sodium channel, that is known as an NaV1.5, the alpha subunit of which is encoded by SCN5A.

So it's an ion channel, and therefore one slide on ion channels since we'll be showing you some ion channel data later. Ion channels are pore-forming membrane proteins. And therefore, as a pore, they allow ions to enter and leave the cell. They therefore have a component of the ion channel that allows it to be either selective or non-selective [INAUDIBLE]. So you can have a sodium-selective channel that won't let potassium in or you can have a non-selective channel that will let both potassium and sodium in.

The one we're talking about is the sodium selective channel, and that's the selectivity pore there. Now, ion channels are gated, they're not always open. And they're gated by one of three major mechanisms. One are ligand gated channels like an acetylcholine gating an acetylcholine channel. Second are the voltage gated ion channels where a change in voltage across the membrane results in a conformational shift and the channel opens. And there is also a favorite of us in the lab that can be mechanically gated, that force can directly change the open probability of a channel.

And something like the channel we'll be speaking about today is gated both by voltage, as well as by force. In the gut, ion channels are king. They're involved in pretty much everything that the gut does, including secretion, motility, as well as neuronal activity. So we've been focused on two predominant cell types that we'll be speaking about today. One is the interstitial cell of Cajal, which is the pacemaker cell of the gut, and the other is the smooth muscle cell.

So one of the ways we've been studying these cells is to dissociate a piece of human gut and then identify the cell of interest and pattern it to record currents. But at the same time, we can also use wider pipettes to actually suck the cell up into a pipette and doing that, do single cell PCR to figure out what genes are giving rise to the transcript that is present in some cells and not in others. As I said, the other way we do this is by actually studying the current itself.

I'm going to spend a lot of time on the slide because of what we're going to be showing later. So if you have a single cell and you put a pipette on it, you can record the change in current that is the result of ion channels opening and closing. So what you can do is set the voltage of the particular cell you're studying to a particular voltage. And you can see here, you can set it at minus 120 to plus 40. At each voltage, you then record the current you get.

So if you look at the blue line here, you can see we set it to this particular voltage. The sodium channel opened, sodium came in. And then if you set it at a different voltage, more depolarized, more sodium comes in. You get to a point where irrespective of how much more you depolarize, you don't get more current. The channel is fully open at that point.

But you also see that despite the fact that the voltage step is this long, the channel did not stay open that long. It inactivated. And that, of course, is a fundamental property of sodium channels, they inactivate rapidly. So you can plot the amount of current at a different voltage, that's the activation, and you can also do a protocol where you hold at a certain voltage and you see how much of an activation you get and you plot. So at a very negative voltage you can get very little inactivation, you can get a lot of inactivation at multiporous voltages.

And then there's a little overlap here, and that's known as the window current, which is where the channel is all the time allowing sodium to enter. So as I said, 16 years ago we were studying these two cell types, and what we noticed when we were studying circular smooth muscle, we saw this current here. Now, it looked like a sodium current, but of course it couldn't have been a sodium current because every textbook said you do not get sodium currents in GI smooth muscle.

And therefore, we spend a year trying to prove it wasn't a sodium current until we finally had to admit defeat, and that meant the fact that it actually was a sodium current. Now, something remarkable about this is that it was only found in the circular muscle layer and not in the longitudinal muscle layer. I'm showing here an L-type calcium current was found in the longitudinal muscle layer and we plotted it here to show the circular muscle layer.

We also then decided to go after the molecular identity and through a series of steps identified that this current was generated by an ion channel, the output subunit of which the gene is SCN5A. And so here you can see the native current in a smooth muscle cell, the native current in interstitial cell of Cajal. This is the current you get if you express the gene in a cellular system. And if you do single cell PCR, you see the product for SCN5A.

Now, this is remarkable. And the reason it's remarkable is because really, SCN5A and NaV1.5 was thought to only be in one place, which is in the heart. In the heart, everybody knew exactly what it did. It was responsible for the upstroke of the QRS complex and mutations in SCN5A result in one of the long QT syndromes, long QT syndrome type 3.

So we were faced with a channel that we didn't know what it did, it appeared to be there. So we said, OK, let's go ahead and look and see if it was changing any of the physiology of the GI tract. And one of the fundamental principles of GI smooth muscle is that if you record some GI smooth muscle, you get an isolation in membrane potential. So it's a slow oscillation in level of seconds that goes up and down, up and down. And that oscillation is driven by the pacemaker cell, the interstitial cell of the heart.

So we recorded from a piece of human jejunum, recorded that slowly, and then added a number of agents to see what happened. And one of the agents that I'm showing you here is lidocaine, which does many things, including blocking sodium channels. And from that, what we were able to do is then take the information we got and model it. And once we modeled it, we said, OK, here's the contribution of the channel. This is the slow wave and by a variety of either modeling pharmacology or physiology, we showed that activation of the channel would depolarize the membrane potential, increase the speed of the upstroke, increase the upstroke amplitude, and decrease the duration of the sodium.

So armed with that information, we began to think, is it possible that there's a role that is not only there in physiology but will also be present in pathophysiology? And that's really where we established the relationship with Mike Ackerman because Mike Ackerman, as most of you know, has a longstanding interest in long QT syndromes. And he has these vast registries of patients who have come to see Mayo and have been shown to have either a mutation of SCN5A or once they find a mutation, you screen the relatives and you find that the relatives have the mutation.

And so what we decided to do was make the assumption, which I think was a pretty safe assumption, that no self-respecting cardiologist would ask a patient about GI symptoms. And therefore, the fact that there were no GI symptoms reported in the literature had absolutely nothing to do with whether they were present or not. And so we said, OK, let's take a questionnaire and send it out to these patients because it's perfect. Their family members will be controls.

We of course were aware of the fact that if you happen to have a disease that could be life-threatening, you can be hyper aware. So as an additional control, we took another channel that was present both in the gut and in the heart and also used that as a secondary control, and that was hERG, which is a potassium channel. So what we find for hERG is that if you have the mutation hERG or not, your GI symptoms are identical. But that was certainly not the case for Nav1.5.

In NaV1.5, if you happen to have the mutation, you are much more likely to have GI symptoms than if you did not. In fact, here's it's 65% plus versus 28%. And so this gave rise to the concept that perhaps a subset of functional abdominal syndromes were due to an ion channelopathy, which would be something that really had been completely unexplored in the gut. And the build to get us to this point is what I just showed you. There was an increased frequency of irritable bowel syndrome, dyspepsia, constipation, and abdominal pain in patients with the mutation SCN5A and also our work in electrophysiology suggesting that not only is it there, but it's doing something.

So we said, OK, now it's time to move towards the patients and begin to see if we can actually find any evidence for this. And at this point, I'm going to ask Art to come up and talk about the second part of the presentation, which is what we've been doing in this area. Thanks, Art.

ARTHUR BEYDER: Well, thanks, everybody, for coming. Thanks, Dr. Farrugia, for this nice background. So as Dr. Farrugia introduced, the point at which this story was now was showing that the channels NaV1.5s were present in the gut, they appeared to be involved in GI physiology, and their involvement in folks with cardiac abnormalities, cardiac conduction disorders, had also correlated with GI abnormalities. And so now the question was, are there such abnormalities in patients with IBS?

So the first part of the study that was done in the lab was a pilot study that had 49 patients who had IBS and the entire gene was sequenced. That revealed a single mutation. This is a missense mutation in one of the extracellular loops in the domain one of the channel. I'll talk a little bit more about the channel structure and such in a second. But that was a G298S mutation. And so two big findings came from that pilot study. I'll show you finding one here.

On the right over here, you'll see a typical set of voltage induced currents for the control setup, so this is yellow currents here. And as you can see here, you get the typical signature sodium current, as Dr. Farrugia had shown. Once that point mutation is introduced-- now, remember this is a single amino acid that's changed in a 2000 amino acid protein-- a very significant drop in conductance is noted. OK? And so this is shown here in a set of IV curves.

And IV is essentially if we were to take voltage at which the current was obtained against that voltage as shown here, one can see that at all voltages studied, significant decrease in peak currents was observed with a single point mutation, which is really a pretty remarkable finding. And so the first finding from this pilot study was that one patient from this cohort had a mutation, and this mutation resulted in a channel with a decreased current.

Now, the gut an electrical mechanical system, and so while we care about the electrical part of it, we certainly care very much about the mechanical components as well. And what I show here is a typical approach to in-vitro testing for mechanical response of electrical components in the GI smooth muscle in ICC and other cells. Essentially, a cell which contains these channels, shown in blue here, is voltage clamped. As before, we put a pipette on there and have a continuum between the inside of the pipette and the cell.

And we simply flow bath solution over the top of the cell, creating a shear stress, which is what we use to model mechanical input. So let me show you what a typical GI smooth muscle ICC will look like when it's voltage clamped and sheared. So shown on the left here is a typical set occurrence, as you've seen before. And just qualitatively, once we apply perfusion, you can see a very significant increase in current.

Now again, for sodium channels, it's an inward current, which means that bigger spikes downward are more current. And so you can see here that the current is bigger and it's also faster. And this is a very robust finding across many, many cells that we've done for years now, showing a perhaps 20% increase in current and a similar increase in speed of the channel operation.

So the second part of the pilot study's findings that were important was the fact that mechanosensitivity is very significantly affected by this point mutation. So again, I shown on the left here a yellow current, which is the control situation. And here you see with the dashed line a significant increase in peak current with shear stress. That's shown here in the bar graph here from baseline up to sheared current. And this was clearly not the case as shown as an example here for G298S.

So for this point mutation, not only was there a peak current that was significantly decreased, but also a significant decrease in mechanosensitivity. And those are the two aspects of molecular function that we were certainly interested in. So this was a pilot study. It identified a single mutation in these 49 patients and specified this to be G28S SCN5A and that had these two findings, decreased peak current and decreased mechanosensitivity. So this is a pilot study, 50 patients, not really controlled by other healthy controls.

And our next big question was, are there more such mutations? Are there SCN5A mutations in the big general population with IBS? And so that has set off a much bigger study, which we had just completed and recently published. So in this study, we had looked at 584 patients with Rome II defined criteria of IBS. This was controlled by 1,380 healthy patients. And we sequenced the entire 27 translated SCN5A exons.

In terms of the IBS cases that were referred to our motility clinic for evaluation, it had demographics similar to what we typically see in Olmstead County. The mean age was about 50 years of age. This was mostly female patients, as we typically see, and distributed as we see here in Olmstead County. The IBS subtypes were also distributed in a typical way we would see IBS distributed with really diarrhea and mixed phenotypes being predominant.

We did find 13 mutations, and I'll discuss this in much more detail in a second. But in terms of demographics here, they're demographically fairly well-matched to the rest of the cohort, with one exception that we noted this to be a more predominant constipation predominant IBS. So more patients had this. So again, now I show the typology of the channel with the 13 mutations pasted on top of it here.

And so 2.2%, which is the rate that we had found from the pilot study, had these unique rare missense mutations. There were an additional 17 polymorphisms of roughly 3% that I don't show here, and I'll discuss those in a second. In terms of the distribution of these mutations, two of them were in transmembrane regions, but the rest were on the intracellular portions of the channel in places that most of them we had no idea what the functional aspects of these mutations should be.

Of interest, eight of 13 had been previously identified by cardiologists to be associated with cardiac conduction disorders, such as LQT, Brugada, SIDS, sick sinus syndrome, sudden death. But very little functional information was available. So this really set us off on a course to try to find out if there are functional abnormalities in terms of channel function that correlate with these mutations. And so in a monster effort here, we had looked at specifically the specific functions of these channels that may be important for GI function.

And so we look at things like peak current. And I show some examples here with white being control few of the mutations shown here with A997T as a blue line that's almost close to zero had significant decreases in peak current. And I'll discuss discussing A997T in one second. We also had found that in several mutations, the voltage dependence was significantly deranged compared to what we see in controls.

And finally, the window current that Dr. Farrugia discussed had been shifted by many of the mutations. So again, window current is this little overlap between the two feet of the activation and inactivation curves. And that area that's shaded here is a place where a small proportion of channels, but a well-defined proportion of channels is constitutively active. It's open. One can see that this is a voltage range where the GI smooth muscle is operating in, and so it's important.

We had also found that in several mutations, there were significantly deranged kinetics. So this top panel will show here compared to the white, which is control, several of the mutations had so much slowed kinetics, as well as inactivation kinetics were shown for a few or the other mutations. Yeah, of course.

AUDIENCE: [INAUDIBLE]. If so, how are these studies done?

ARTHUR BEYDER: Heterozygous.

AUDIENCE: [INAUDIBLE].

ARTHUR BEYDER: Yes.

AUDIENCE: --about the mutant, but there was a wild type along with it [INAUDIBLE]. Is there one alpha subunit for each of these channels, or are there more [INAUDIBLE].

ARTHUR BEYDER: For SCN5A there is one alpha. There are sodium channels. Now, there are papers out there and the cardiology folks have looked very closely at sodium channel function. And so there are papers looking at heterozygosity with one of the genes being abnormal. There are very clearly functional consequences from doing so. Yes. For these mutations, it's heterozygous.

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AUDIENCE: The sample?

ARTHUR BEYDER: This is looking at this protein. So this DNA that's altered with this missense mutation that we identify expressed in the expression cell system that we use.

AUDIENCE: All right. So you know for sure it's that one and not the wild thing.

ARTHUR BEYDER: Absolutely, yeah. So we show here that some of the mutations had significant abnormalities in inactivation rates. And so to put a lot of work onto a single table without any specific bias here, we do this here. And more specifically here colored in blue are the loss of function findings that we had found for several of the mutations and red is the gain of function.

Overall, of the 13 mutations that we had studied, only three ended up not having any functional changes, one had a gain of function, and the majority had a loss of function phenotype in vitro with patch [INAUDIBLE]. So I show this here on the typology figure again. And the most impressive thing for us was that 3/4 of the mutations were functionally abnormal and the majority of these were a loss of function mutation.

And this was really a fairly revealing finding. As I said before, we did find some polymorphisms and those had been previously looked at in the electrophysiology realm of the cardiologists. All but one had functional abnormalities and all but two had been associated in one way or another with some of the cardiac conduction disorders, suggesting that there may be some pathogenesis that carried along with these polymorphisms. So now we had a large set of patients the genes of which were genotyped and studied closely controlled by a large cohort of healthy patients.

But as Dr. Farrugia is saying, the information on their functional bowel diseases is not often available. We had collaborated with Dr. D'Amato and his group at Karolinska, who actually have huge cohorts of patients with IBS. And they control it by patients that they know specifically do not have IBS. They have analyzed a large GWAS study showing here that there was a signal for SCN5A in their GWAS. So they repeated the analysis in four of the multinational cohorts.

And so across large cohorts now with thousands of patients, it does appear that SCN5A certainly correlates and from our standpoint is functionally abnormal when we find mutations. So one of the mutations that had really jumped off the page at us with the multiple functional abnormalities here is this A997T. So now we have this mutation and that is on an intracellular linker between domains two and three, really in an area where nobody has previously identified to be important for the protein.

But as you'll see, that doesn't always make the biggest difference. So let me show you some of the electrophysiology data and we'll move to some of the patient data that we have. So this is a control curve that we see. This is, again, expressed in the hex cells, which is our typical expression system. And what you'll see here is that the channel with A997T mutation introduced to it will have a very significant drop in peak currents, and that's shown here.

In IV curves which we've shown before, there's a very significant rightward shift in voltage dependence of the currents and a significant slowing of the activation. So this is how fast does the upstroke get to the top with A997T mutations. So I put the two traces on top of each other with the orange being A997T compared to the yellow, which is control. And you can see that there's fairly dramatic changes to its function in vitro setting in an expression system.

So the next question was, is there any way to be able to rescue it? And Pete Strege, who is really an electrophysiologist extraordinaire in the lab, had spent some time and had gone through the literature and had seen that drugs such as mexiletine have been previously used to restore function to some of the dysfunctional sodium channels. What he'd done is essentially incubate the cells with the mexiletine, expressing A997T mutation, and had shown that unlike the previous figure, there's very significant restoration occurrent, as you can see here; normalization of the kinetics; and almost normalization of the IV traces.

And so that was really an impressive finding. So this was with no mexiletine here. And with addition of mexiletine, we can clearly see that there is near normalization of the channel's function. But what's really interesting is what this has to do with the patient or what can we do. And so the mutation A997T in the sodium channel we're talking about belonged to a 65-year-old Caucasian female. So she's got many years of IBS-C.

And in fact, if you ask her, she'll tell you that she never remembers not having constipation and abdominal pain. She has had no significant past medical history, at least something that would contribute. She had a negative physical exam, has been looked at closely by multiple specialists, and very significantly has had an extensive negative workup that included CT of the abdomen and pelvis that was negative and importantly, anorectal manometery and normal defecography, which preclude a contribution from pelvic floor dysfunction.

What we see here is a transit study that's a technique developed by Dr. Camilleri and his group showing scintigraphic evidence of how the contents move through the colon. We show here that in a normal setting, the white matter labeled here sits roughly in the middle of the colon right here after 24 hours, so geometric centered at about two. And at 48 hours, everything moves over closer to the left side here and the geometric center becomes a 3.7. Just an arbitrary number, but this is what a typical normal situation looks like.

So for our patient here, a very significant slowing in transit is shown here. At 24 hours, we have a geometric center of 1.3 and at 48 hours, this is at 2.0. And so there's very clearly a significant slowing of colonic motility. And perhaps even more impressive was the fact that her bowel habits going back about five weeks' worth had normal bowel movements roughly once per week with some small hard bowel movements that she had had intermittently alongside.

So the question was, can we treat her with mexiletine? Now, this is not something that we obviously routinely do in GI practice because it's a cardio drug and requires EKGs and monitoring. We were really fortunate to be supported by the CCAT Stimulus Grant and the folks over in CRU that allowed us to put this patient onto 24/7 telemetry, so it's an n of one study. She came in for 24/7 telemetry and titrated mexiletine over five days.

We started with a pretty minimal dose and got up to what a clinically relevant dose would be. And so this shows the study protocol. And we had interacted with her at one and two here, as well as after the study, as well as with a bowel habits diary before and after the study. What was really the most impressive thing for us after all was said and done is the response that she had to mexiletine treatment with respect to her IBS-C.

Her abdominal pain did significantly improve, but really impressively was the amount of bowel movements. So this is at seven bowel movements per week, which basically makes one bowel movement per day, which most of us would consider a normal frequency. And this had tapered down to roughly her baseline status over five to six weeks. So three months later in following up with her, the movements and symptoms were back to baseline. She did restart Miralax with a fair response, but she is considering returning for potentially a low-dose chronic mexiletine therapy.

Now, of course, this is not something to imply that mexiletine is useful for every patient with IBS-C. But in her particular case, what we're seeing is that she had a specific mutation in a sodium channel, a specific functional abnormality and both in vitro and in vivo had been helped by mixeletine. So specific in a patient-oriented response. So let me summarize a few of these things here.

In terms of IBS and SCN5A, we know from Dr. Farrugia's part of the presentation here that the SCN5A is encoding an alpha subunit of a sodium channel NaV1.5. It is present in the human GI tract, it's expressed in the smooth muscle cells and interstitial cells of Cajal. In the smooth muscle strips, the blockade of that channel does affect the electrical activity of the smooth muscle strips, and so that's important.

We also know that SCN5A is important in IBS. And so we show that SCN5A mutations do associate with increased prevalence of IBS, but also show that mutations and polymorphism occur more frequently in IBS. We also know now that NaV1.5 is functionally abnormal if disrupted by IBS-related mutations, as we show here. And so the mutations in SCN5A may account for 2.2% of IBS patients. Now, that sounds like a small number, but given the prevalence of the disorder, we're talking potentially about up to two million people that may be affected with an ion channelopathy responsible for their IBS.

In terms of treatment in this pilot study with this patient that we had, this essentially shows that the A997T mutation has a significant loss of function associated with significant colonic transit delay, and both in vitro and in vivo function were restored with mexiletine. While, again, this is not to endorse mexiletine to be used for IBS in general, certainly not for IBS-C, what this does do is it opens the door for the potential disease-modifying therapies as opposed to the symptom-modifying therapies which we've all been doing in the field of IBS.

A very large group of people have contributed to this work, and mine was probably one of the smaller parts. Dr. Farrugia's group, it's been really an honor to work as a part of a group and interactions have just been amazing. Pete had done most of the electrophysiology work and really has done the mutagenesis and Cheryl makes the lab go around. The gastroenterology allergy group has helped us tremendously with the patient-centered studies.

We did collaborate with Dr. Ackerman and his group in cardiology. And the work from Dr. D'Amato and Karolinska Institut had helped look at the multi-national cohorts. And we appreciate the funding and support and really your attention. Thank you.

[APPLAUSE]